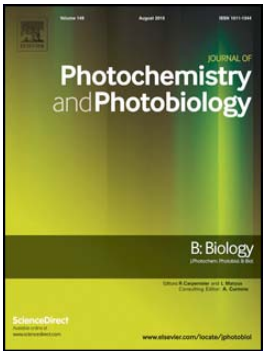


Potent Acetylcholinesterase Inhibitors: Synthesis, Biological Assay and Docking Study of Nitro Acridone Derivatives

Mehtab Parveen, Afroz Aslam, Shahab A.A. Nami, Ali Mohammed Malla, Mahboob Alam, Dong-Ung Lee, Sumbul Rehman, P.S. Pereira Silva, M. Ramos Silva

PII: S1011-1344(16)30205-6
DOI: doi: 10.1016/j.jphotobiol.2016.05.028
Reference: JPB 10401

To appear in:

Received date: 25 March 2016
Revised date: 15 May 2016
Accepted date: 30 May 2016

Please cite this article as: Mehtab Parveen, Afroz Aslam, Shahab A.A. Nami, Ali Mohammed Malla, Mahboob Alam, Dong-Ung Lee, Sumbul Rehman, P.S. Pereira Silva, M. Ramos Silva, Potent Acetylcholinesterase Inhibitors: Synthesis, Biological Assay and Docking Study of Nitro Acridone Derivatives, (2016), doi: 10.1016/j.jphotobiol.2016.05.028

Potent Acetylcholinesterase Inhibitors: Synthesis, Biological Assay and Docking Study of Nitro Acridone Derivatives

Mehtab Parveen*[,1], Afroz Aslam[1], Shahab A. A. Nami[2], Ali Mohammed Malla[1], Mahboob Alam[3], Dong-Ung Lee[3], Sumbul Rehman[4], P. S. Pereira Silva[5], M. Ramos Silva[5]

[1]Department of Chemistry, Aligarh Muslim University, Aligarh 202002, India
[2]Department of Kulliyat, Faculty of Unani Medicine, Aligarh Muslim University, Aligarh 202002, India
[3]Division of Bioscience, Dongguk University, Gyeongju 780-714, Republic of Korea
[4]Department of Ilmul Advia, Faculty of Unani Medicine, Aligarh Muslim University, Aligarh 202002, India
[5]CEMDRX, Physics Department, University of Coimbra, P-3004-516 Coimbra, Portugal.

*Email: mehtab.organic2009@gmail.com
Tel: +91 09897179498

Abstract

The reaction of *o*-halobenzoic acid with aniline derivatives and their subsequent cyclization reaction yielded the acridone derivatives. The series of nitro acridone derivatives were prepared by Ullmann condensation in presence of copper as catalyst and were characterized by FTIR, ^{1}H, ^{13}C NMR and Mass spectra. The structure of 5-nitro-(2-phenyl amino) benzoic acid **(4)** was confirmed by X-ray crystallography and was found to crystallize in *P21/c* space group. The *in vitro* efficacy of the compounds for their acetylcholinesterase (AChE) and antimicrobial inhibitory activities have been evaluated against the standard drugs Ampicillin and Gentamicin against gram positive and gram negative bacteria. 1,7-dinitroacridone was found to be the most potent AChE inhibitor (IC_{50} = 0.22 µM). Moreover, the compounds have been screened for their antioxidant activity using the DPPH assay. Also, docking study results were found to be in good agreement with the results obtained through *in vitro* experiments. The docking study further predicted possible binding conformation.

Keywords: Acridone; Acetylcholinesterase; Antioxidant activity, Docking study.

1. Introduction

Chemical modification of bioactive components is one of the most commonly employed approaches in drug discovery research because of its promising results [1]. The occurrence of heterocyclic derivatives in bioactive natural products and pharmaceuticals has made them significant synthetic targets. Acridones are an exclusive kind of heterocycle, a subclass of acridines with a basic structure consisting of 9(10H)-acridone which is present in a large number of natural products and synthetic compounds that are known as multi-targeted agents with biomedical perspective. Acridone-based derivatives were first pursued for their antimicrobial activity against bacteria, parasites and fungi [2,3]. It is an important organic compound bearing tricyclic nitrogen containing ring and was first used in 19th century against malaria [4]. Acridone consists of two benzene rings fused together having a keto group and a nitrogen atom at 9th and 10th position, respectively, resulting in a planner structure. This chemical scaffold bears a wide range of biological activity including anti-cancer [5], anti-malarial [6], anti-viral [7,8] and modulation of multi-drug resistance (MDR) [9-11]. On the molecular level, the planner structure of acridone molecule facilitates its interaction with nucleotides leading to intercalation with DNA and RNA strands. Interaction of DNA with various biologically important organic molecules has been extensively reviewed in the literature [12-14]. A series of 1-amino thioacridones were designed as DNA intercalating agents with covalent bond formation potential [15]. The intercalation of acridone molecule with DNA and RNA is primarily based on its π-π stacking interactions with base pairs of double-stranded nucleic acids. These interactions of acridine analogs have also been confirmed by X-ray crystallography revealing ring overlap between acridine ring and base pairs of nucleic acid [16]. The biological consequence of these interactions is the disruption of DNA functions in the cells, serving as a prerequisite for effective anti-cancer activity of acridine analogs [17]. Moreover, acridone molecule also possesses hydrophilic and lipophilic

balance which facilitates its transverse through biological membranes to reach into the nucleus, exerting their action [18].

Acridone is highly fluorescent and stable against photo degradation, oxidation, and heat [19,20]. It is a small molecule with no charge. Several acridone derivatives have been used as fluorescent labels for peptides [21], amino acids [22], antibodies [23] and substrates for catalysis [24]. A series of acridone linked with nitro group have been developed as potential anticancer agent, among which nitracrine appeared to be very potent cytotoxin and 4-nitroacridone was found to be the best mycophenolic acid.

There are several methods for the synthesis of acridone including the Bernthsen reaction [25] which is one of the oldest methods for the synthesis of acridones. The most well known methodologies for the synthesis of acridone are reported by Meyer [26], Koller [27], besides several new methods have also been cited in the literature [28-33]. In 1937, Tanasescu and Suciu [34] described a synthetic approach to acridones through rearrangement of benzo[*c*]isoxazoles (anthranil) in the presence of concentrated Sulphuric acid containing nitrous acid as catalyst. Keeping in view of these and in continuation of our research on biologically active molecules, we hereby report the synthesis and characterization of some acridone derivatives using Ullmann condensation [35] between substituted *o*-halobenzoic acid and aniline derivatives followed by Friedel-Crafts acylation. The compounds (**8-10**) have also been screened for their plausible *in vivo* antimicrobial and antioxidant activities. The synthesized compounds (**8-10**) were further subjected for their docking studies to explore the binding pattern of the compounds.

2. Result and discussion

2.1. Chemistry

The substrate *N*-phenylanthranilic acids (**4-6**) used as starting compounds (Scheme 1) were prepared through condensation of substituted *o*-halobenzoic acid with an excess of the appropriate substituted aromatic amine. To this mixture $POCl_3$ was added and refluxed for 1

h and stirred for another 2 h in presence of 1 M HCl to obtain the final substituted acridone derivatives (**8-10**). Its purity has been ascertained on the basis of TLC. Moreover, the precursor has been subjected to spectral (IR, ^{1}H NMR, ^{13}C NMR and MS studies) analysis. The compounds (**8-10**) have been obtained in good yield. The selected IR bands of the synthesized compound provide useful information regarding the structure of the acridone derivatives. The strong absorption band at 1635 (**8**), 1632 (**9**) and 1620 (**10**) cm^{-1} may be assigned to characteristic cross conjugated (C=O) group while the closely spaced band observed at 1545, 1337 (**8**), 1532, 1350 (**9**) and 1535, 1337 (**10**) may be ascribed to the nitro group implying the formation of acridone derivatives (**8-10**). In the ^{1}H NMR spectrum, the assignments of the signals are based on the chemical shift and intensity pattern. The spectra of the compounds (**8-10**) exhibited a singlet at δ 12.35 (**8**), 12.31 (**9**) and 12.85 (**10**) integrating for N-H protons. A sharp singlet at δ 8.42 and 8.56 ppm (**8** and **10**) has been accredited to H-1 and H-8. This prominent downfield shift of H-1 and H-8 protons is supposed to be due to their hydrogen bonding interaction with the neighbouring carbonyl group, which causes them to resonate at elevated δ values.

The ^{13}C NMR signals are found to be in corroboration with the proposed structure of the compound (**6-8**). The compounds exhibited carbonyl carbon (C=O) signal 169.49 (**8**), 176.14 (**9**) and 170.25 ppm (**10**) respectively. A group of signals resonating in between 151.83-111.32 (**8**), 148.88-110.20 (**9**) and 149.80-113.65 ppm (**10**) are assigned to aromatic ring carbons. However, compounds (**8-10**) showed characteristic molecular ion peaks, MS (ESI) at m/z: 240 (**8**), 240 (**9**) and 285 (**10**) respectively which were in good concurrence with the proposed structures.

2.2. *Crystal structure*

Single crystals appropriate for analysis were obtained by slow evaporation of methanol at room temperature. X-ray crystallographic analysis of the compound (**4**) reveals that it crystallizes in the monoclinic crystal system with space group *P 21/c*. The asymmetric unit of

the compound (**4**) has been shown in **Fig. 1**.The asymmetric unit showed that molecule crystallize as its sodium salt with three molecules of hydration. The hydrated sodium atom appears to bind with oxygen atom of the nitro group *via* electrostatic interaction. This type of interaction has been previously reported by Yazicilar *et.al*. [36]. Pertinent crystallographic data for compound (**4**) has been summarized in **Table 1**. It can be seen from the three dimensional (3D) framework of compound (**4**) that it is stabilized *via* an intricate array of hydrogen bonding. The three water molecules play an important role in stabilizing 3-D structure, forming extensive hydrogen bonding between carboxylic oxygen and water molecules (COO....H-O) as depicted in **Fig. 2**.

2.3. Acetylcholinesterase Inhibition (AChEI) Docking Studies

The molecular docking studies were carried out to support the experimental AChE inhibition results. The synthesized molecules were subjected to docking study using PatchDock, Discovery studio 4.0 Client and iGEMDOCK softwares to predict the binding mode of compounds towards target enzyme (PDB:3NM8). The docking results and their scoring functions presented crucial information regarding the orientation of the compounds and the strength of the non-covalent interaction (binding affinity) between ligand and receptor in the binding cavities. On the basis of docking simulations, the strong binding affinity of compound **10** with AChE can be explained on the basis of hydrogen bonding/secondary interactions as well as orientation and electronic features of the substituents towards the active site of the target enzyme **Fig. 3.** The hydrogen bonds interacted with amino acids of the enzyme are in good agreement with the predicted binding affinities obtained by molecular docking studies as verified by AChE inhibition activity data where compound **10** was found to be most potent AChE inhibitor with IC_{50} value of 0.22 µM in close proximity to standard drug Tacrine with IC_{50} value of 0.20 µM (**Table 2)**. The improved acetylcholinesterase inhibition of compound **10** in comparison to the other products **8** and **9** can be explained on

the basis of mode of interaction among the substituents at the molecular skeleton and amino acids of enzyme acting as receptor. The two nitro groups at 1 and 7-position of acridinone moiety could interact with THR31, LYS89 and SER 88 of the enzyme's amino acids through hydrogen bonds. The amino acids of enzyme around the cavity can have a contact with ligands making the long distance secondary bonds. In case of compound **8** and **9**, the nitro group of compound **9** at position 1 makes intermolecular hydrogen bond with adjacent keto group of the molecule, while nitro group of compound **8** is not in proper orientation in order to make effective bonds with amino acids. Therefore, the available site of ligand either engaged in the formation of secondary bond within the molecule or not in proper orientation for making additional non classical bonds. However, compound **10** is believed to have a better pharmacological activity among other derivatives **8** and **9**. The increased activity of compound **10** among the three compounds could be explained on the basis of obtained docking score, a representative of the binding affinity of ligand to receptor. The binding score of compounds **10**, **9** and **8** obtained are -89.12, -88.66 and -81.04 (kcal/mol), respectively (**Fig. 4)**. The mentioned docking score clearly depicts that binding energy of compound **10** (-89.12) is higher than that of **8** and **9** implying stronger interactions between compound **10** and amino residues of target enzyme. Therefore, compound **10** is an effective blocker for AChE. Additionally, the skeleton of acridinone also plays an important role in stabilizing the ligand-receptor complex by pi (π)-interaction with amino acids of the target enzyme as shown in **Fig. 5**. The π- π interactions between aromatic rings of the compound with amino acids of the protein further stabilize the orientation of the molecule into cavity of receptor. From the combined table and chart shown in **Fig. 4** it is evident that compound **10** showed an effective activity against AChE and revealed better affinity to receptor and showed maximum docking score as it was buried well inside the cavity of the target enzyme. Ligand map (**Fig. 6)** was used to explain the in-depth interactions between the docked molecule and active site of the

protein. Number of non-covalent interactions such as hydrophobic, hydrogen bonding and van der Waals forces and their pattern were matched with the help of diagrams automatically generated by MMV software. Ligand map gives a set of divergent intuitive future and are also helpful in knowing the secondary interactions pattern after docking. Moreover, geometrical descriptors of compounds **8**, **9** and **10** were also used to show the extent of the surface interaction and can be applied for predicting pharmacological and toxicological activities of molecules on the basis of structure activity relationship (**Fig. 7**). The values of descriptors obtained using ChemAxon software were employed to establish the idea of active compound among the studied compounds. In general, more projection area implies more interaction to the receptors as shown in **Table 3**.

2.4. Antimicrobial activity

The *in vitro* antimicrobial activity of compounds (**8-10**) were studied against *Staphylococcus aureus* (ATCC 29213), *S. epidermedis* (MTCC 435), *Streptococcus mutans* (ATCC 25175), *S. pyrogenes* (MTCC 435), *S.viridans, Corynebacterium diphtheria,* (gram-positive bacterial strains) *E. coli* (ATCC 25922), *P. aeruginosa* (MTCC 424) and *Proteus vulgaris* (MTCC 426) (gram-negative bacterial strains) by disc diffusion method. The antibacterial activity of each compound was compared with Gentamicin and Ampicillin drug. The disc poured in DMSO was used as negative control. The antibacterial data obtained has been reported in **Tables 4** and **5** and the values are reported in terms of the zone of inhibition in millimetre (mm). Among the synthesized compounds, compound (**10**) was found to be active against all the Gram positive bacterial strains except *Streptococcus pyrogenes*. It can be inferred from the data reported in **Table 4**, that compound (**10**) displayed potential inhibition zones 18 and 14 mm against *Staphylococcus aureus* and *S. viridians* bacterial strains. All the synthesized compounds (**8-10**) were found to be more active against Gram positive as compared to Gram negative bacterial strains (**Table 5**). Compound (**9**) displayed highest activity against *Proteus*

vulgaris with zone of inhibition 16 mm in vicinity to standard drug Gentamicin with zone of inhibition 18 mm for the same bacterial strain.

2.5. Antioxidant study

The synthesized acridone derivatives (**8-10**) were subjected to free radical scavenging activity by DPPH method. This model of scavenging activity by DPPH radical is extensively applied to evaluate antioxidant activity conveniently as compared with other methods. The odd electron in the DPPH free radical gives a strong absorption band at 517 nm, which is purple in color. This property makes it suitable for spectrometric studies. The DPPH assay has often been used to estimate the antiradical activity of a given antioxidant. The free radical scavenging capabilities of the compounds were measured in term of hydrogen donating of free radical scavenging ability after adding methanolic solution of DPPH to the sample solution having different concentrations. The extent of decolourizing is indicative of antioxidant behaviour of a particular compound. Ascorbic acid was used as the reference compound. All the tests were performed in triplicate. The compound (**10**) showed the highest IC_{50} value (27.80 µ/mL) followed by compounds (**8**) and (**9**) respectively and results are reported in **Table 6**.

3. Experimental

3.1. Synthesis and characterization

Melting points were determined on a Kofler apparatus and are uncorrected. Elemental analysis (C, H, N) were conducted using Carlo Erba analyzer model 1108. The IR spectra were recorded with Shimadzu IR-408 Perkin-Elmer 1800 (FTIR) and its values are given in cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were run in DMSO-*d6* on a Bruker Avance-II 400 MHz instrument with TMS as internal standard. Chemical shifts are reported in ppm (δ) relative to the TMS. Mass spectra were recorded on a JEOL D-300 mass spectrometer. Thin layer chromatography (TLC) plates were coated with silica gel G and exposed to iodine vapours to check the homogeneity as well as the progress of reaction. Sodium sulfate or magnesium

sulfate (anhydrous) was used as a drying agent. All the reagents were purchased from Sigma-Aldich (India) which were of analytical grade and used without further purification.

*3.2. General procedure for the synthesis of substituted N-phenylanthranilic acid derivatives (**4-6**)*

The mixture of substituted *o*-halobenzoic acid (**1**) (0.04 mol) and substituted aniline (**2**) (0.08 mol) were refluxed in DMF in presence of anhydrous sodium carbonate (1 mol%) and copper powder (3 mol%) for 4 hrs. After completion of the reaction as evident from thin layer chromatography (TLC), the reaction mixture was cooled to room temperature and then slowly added with shaking to a HCI and water (1:1) solution (100 mL). The reaction mixture was left to stand overnight. The solid precipitated out was filtered, washed with boiling water and then crystallized with appropriate solvent to yield substituted *N*-phenylanthranilic acid (**4-6**) **Scheme 1.**

4.2.1 5-nitro-(2-phenyl amino) benzoic acid (**4**)

Crystallized from methanol-acetone as an orange-yellow color solid; Yield: 90%, m.p. >300 ^{o}C. Anal.Calc. for $C_{13}H_{10}N_2O_4$; C, 60.47; H, 3.90; N, 10.85; O, 24.78; found: C, 60.52; H, 4.72; N, 8.24; O, 24.76. IR (KBr) cm^{-1}: 3067 (N-H), 1590-1433 (C=C$_{aromatic}$), 1523-1329 (NO_2), 1618 (C=O, acid carbonyl) ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.58 (s, 1H, N-H, D_2O-exchangeable), 8.82 (s, 1H, COOH), 8.81 (s, 1H, H-1), 8.03 (d, 1H, H-3), 7.40 (dd, 2H, H5-H9), 7.26 (dd, 2H, H6-H8) 7.15 (d, 1H, H-4), 7.11 (m, 1H, H-7). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 169.49 (C-1), 151.83 (C-13), 139.51 (C-6), 136.14 (C-3), 129.54 (C-4), 128.58 (C-7, C-9), 126.90 (C-2), 124.08 (C-9), 122.24 (C-7, C-11), 111.32 (C-5, C-12). MS (ESI): (m/z) 334.26 $[M+H]^{+\bullet}$ ($C_{13}H_{15}N_2NaO_7$).

4.2.2. 2-(3-nitrophenyl) amino benzoic acid (**5**)

Crystallized from methanol-acetone as orange red color solid; Yield: 85%, m.p. 260-265 ^{o}C. Anal.Calc. for $C_{13}H_{10}N_2O_4$; C, 60.47; H, 3.90; N, 10.85; found: C, 60.52; H, 4.72; N, 8.24; O, 42.76. IR (KBr) cm^{-1}: 3191-3079 (N-H), 1582-1455 (C=C$_{aromatic}$), 1529-1357 (NO_2), 1604

(C=O, acid carbonyl) ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.23 (s,1H, N-H, D_2O-exchangeable), 8.76 (s, 1H, COOH), 7.99 (d, 1H, H-4), 7.97 (d, 1H, H-5), 7.89 (d, 1H, H-1), 7.65 (d, 1H, H-7), 7.18-7.51 (m, 2H, H-2, H-3), 7.47 (m, 1H, H-6). ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 168.65 (C-1), 148.70 (C-9, C-13), 141.51 (C-10), 132.05 (C-2), 130.56 (C-7), 129.45 (C-4), 128.50 (C-6), 123.67 (C-3), 121.76 (C-8), 118.68 (C-12), 113.93 (C-5), 108.64 (C-11). MS (ESI): (m/z) 258.23 $[M+H]^{+\bullet}$ ($C_{13}H_{10}N_2O_4$).

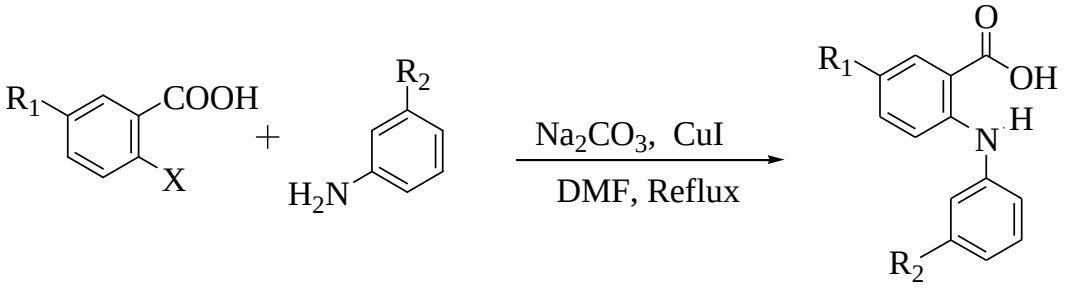
4.2.3. 5-nitro-(2-(3-nitrophenyl) amino) benzoic acid (**6**)

Crystallized from methanol-acetone as dark green color solid; Yield: 87%, m.p. 290-295 ^{o}C. Anal.Calc. for $C_{13}H_9N_3O_6$; C, 51.49; H, 2.99; N, 13.86; O, 31.66; found: C, 60.52; H, 4.72; N, 8.24; O, 31.64. IR (KBr) cm^{-1}: 3277-3091 (N-H), 1535-1440 (C=$C_{aromatic}$), 1526-1342 (NO_2), 1678 (C=O, acid carbonyl) ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.75 (s, 1H, N-H, D_2O-exchangeable), 8.75 (s, 1H, COOH), 8.59 (s, 1H, H-1), 8.37-7.28 (dd, 1H, H-3, H-4), 8.06 (d, 1H, H-5), 7.73 (d, 1H, H-7), 7.69 (s, 1H, H-9), 7.63 (m, 1H, H-6). ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 168.35 (C-1), 151.07 (C-13) 148.58 (C-7), 139.65 (C-11), 137.64 (C-3), 130.79 (C-4, C-9), 129.41 (C-2), 126.70 (C-10), 119.46 (C-5), 117.59 (C-8), 113.58 (C-6), 112.36 (C-12). MS (ESI): (m/z) 303.05 $[M+H]^{+\bullet}$ ($C_{13}H_9N_3O_6$).

*3.3. General procedure for the cyclization of N-phenylanthranilic acid derivatives to substituted acridones **(8-10)***

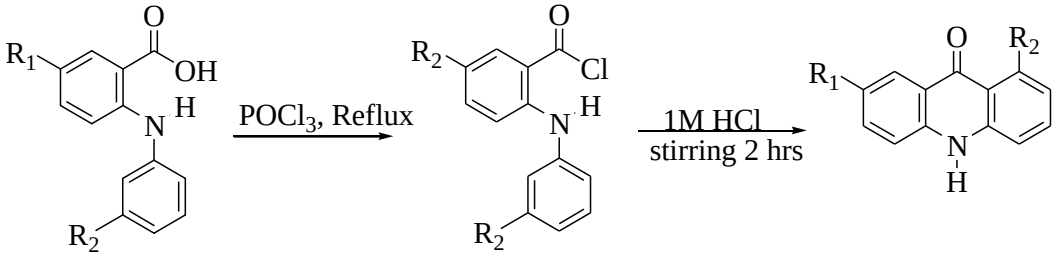
Substituted *N*-phenylanthranilic acid (**4-6**) (7.77 mmol) synthesised in the first step were dissolved in $POCl_3$ (50 mL) and refluxed for 1 h. After completion of the reaction as evident from TLC, the excess of $POCl_3$ was recovered *via* vacuum distillation and the solid 9-chloro intermediate product (**7**) was obtained. To a flask containing the 9-chloro intermediate was added 30 mL of 1 M HCl. The mixture was mildly heated over a heating mantle with stirring for 2 h. The reaction mixture was cooled and the product was extracted with dichloromethane, dried over magnesium sulfate and concentrated. The crude product

obtained was washed with suitable solvents, filtered, dried and crystallized from appropriate solvents to afford pure target products (**8-10**) respectively as shown in **Scheme 1.**



	R1	R2	X
1	NO_2	H	Cl
2	H	NO_2	Br
3	NO_2	NO_2	Cl

	R1	R2
4	NO_2	H
5	H	NO_2
6	NO_2	NO_2



4-6 **7**

	R_1	R_2
8	NO_2	H
9	H	NO_2
10	NO_2	NO_2

Scheme 1 Synthetic route for the preparation of acridone analogs

3.3.1. 2-nitroacridinone (**8**)

Crystallized from methanol-acetone as yellow color solid; Yield: 90%, m.p. >300 ^{o}C. Anal.Calc. for $C_{13}H_8N_2O_3$; C, 65.00; H, 3.36; N, 11.66; O, 19.98; found: C, 59.98; H, 3.34; N, 11.64; O, 19.96. IR (KBr) cm^{-1}: 3272-3022 (N-H), 1582-1477 ($C=C_{aromatic}$), 1545-1337 (NO_2), 1635 (C=O) ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.35 (s, 1H, N-H, D_2O-exchangeable), 8.90 (d, 1H, H-5), 8.41 (d, 1H, H-3), 8.19 (d, 1H, H-1), 7.70 (d, 1H, H-4),

7.62 (m, 1H, H-8), 7.56 (m, 1H, H-6), 7.34 (m, 1H, H-7). ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 176.36 (C-9), 144.40 (C-13), 140.60 (C-12), 140.48 (C-2), 134.51 (C-6), 127.33 (C-3), 126.02 (C-8), 122.96 (C-1), 122.83 (C-7), 120.68 (C-11), 119.04 (C-14), 118.84 (C-4), 117.95 (C-5). MS (ESI): (m/z) 240.05 $[M + H]^{+\bullet}$ ($C_{13}H_8N_2O_3$).

3.3.2. 1-nitroacridinone (**9**)

Crystallized from methanol-acetone as brown color solid; Yield: 87%, m.p. 270-275 ^{o}C. Anal.Calc. for $C_{13}H_8N_2O_3$; C, 60.47; H, 3.90; N, 10.85; O, 19.98; found: C, 60.52; H, 4.72; N, 8.24; O, 19.96. IR (KBr) cm^{-1}: 3241-3096 (N-H), 1545-1468 (C=C$_{aromatic}$), 1532-1350 (NO_2), 1632 (C=O) ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm):12.31 (s, 1H, N-H, D_2O-exchangeable), 8.65 (d, 1H, H-2), 8.26 (d, 1H, H-4), 7.90 (d, 1H, H-5), 7.72 (m, 1H, H-3), 7.61 (d, 1H, H-8), 7.45-7.29 (m, 2H, H-6, H-7). ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 176.16 (C-9), 148.88 (C-1), 141.53 (C-11), 140.48 (C-13), 134.42 (C-3), 133.28 (C-6), 126.07 (C-8), 125.73 (C-4), 123.31 (C-12), 122.25 (C-7), 120.93 (C-14), 117.67 (C-5), 115.10 (C-2). MS (ESI): (m/z) 240.05 $[M+H]^{+\bullet}$ ($C_{13}H_8N_2O_3$).

3.3.3. 1,7-dinitroacridinone (**10**)

Crystallized from methanol-acetone as dark green color solid; Yield: 85%, m.p. 294-296 ^{o}C. Anal.Calc. for $C_{13}H_7N_3O_5$; C, 54.74; H, 2.47; N, 14.73; O, 28.05; found: C, 54.72; H, 2.45; N, 14.71; O, 2.03, IR (KBr) cm^{-1}: 3273-3026 (N-H), 1525-1470 (C=C$_{aromatic}$), 1535-1337 (NO_2), 1620 (C=O) ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.85 (s, 1H, N-H, D_2O-exchangeable), 8.90 (d, 1H, H-2), 8.56 (m, 1H, H-8), 8.75 (d, 1H, H-6), 7.97 (m, 1H, H-3), 7.82 (d, 1H, H-5), 7.64 (d,1H, H-4). ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 176.20 (C-9), 149.86 (C-1), 147.10 (C-12), 144.23 (C-14), 141.34 (C-7), 135.20 (C-3), 128.45 (C-6), 124.92 (C-4), 123.23 (C-8), 122.40 (C-11), 119.90 (C-13), 118.45 (C-5), 115.21 (C-2). MS (ESI): (m/z) 285.04 $[M+H]^{+\bullet}$ ($C_{13}H_7N_3O_5$).

3.4. Crystal structure determination of compound (**4**)

Single crystal X-ray data of compound **(4)** was collected at 100 K on a Bruker SMART APEX CCD diffractometer using graphite monochromated MoKα radiation (λ = 0.71073Å). The linear absorption coefficients, scattering factors for the atoms and the anomalous dispersion corrections were taken from the International Tables for X-ray Crystallography [37].The data integration and reduction were carried out with SAINT software [38]. An empirical absorption correction was applied to the collected reflections with SADABS, and the space group was determined using XPREP [39]. The structure was solved by direct methods using SHELXTL-97 and refined on F2 by full-matrix least-squares using the SHELXL-97 [40] program package. All non-hydrogen atoms were refined anisotropically. Pertinent crystallographic data for compound **(4)** is summarized in **Table 1**.

3.5. Molecular Docking

The three-dimensional crystal structure of receptor was taken from Protein Data Bank (pdb). The retrieved protein (PDB: 3NM8) was loaded in the Molegro virtual docker (MVD) [41] with the removal of all water molecules. The standard Molegro algorithm was utilized for rendering the missing charges, protonations states and assigning of polar hydrogens to the receptor for achieving reliable potential binding sites in the receptor. All the compounds were drawn using ChemDraw Ultra version 12.0 and structures were analyzed using Chem Draw Ultra3D software. These structures were energetically minimized using MM2 force field with RMS gradient set to 0.0001 and finally saved as pdb file for docking process. PatchDock [42], Discovery studio 4.0 Client [43] and iGEMDOCK [44] softwares were used to sort out best molecular docking poses of ligand (compound)-receptor interactions, perform visualization of docked ligands and illustration of basic features of the docked interface and compute energy calculation of docked ligands, respectively. In all cases, the program's default parameters were used. The scoring function obtained by GEMDOCK [44], which is based on a piece-wise linear potential. GEMDOCK generates and calculates the different

interactions involved between receptor and molecules, i.e., electrostatic, hydrogen and van der Waals interactions. The PARS [45], a web server, was used to predict the cavities on the receptor for the successful docking at binding sites. These locations may cause a regulatory effect upon binding of the receptor when interacts with the guest.

3.6. Biological activity

The biological activity profile of the synthesized compounds (**8-10**) as acetylcholinesterase (AChE) inhibitor was assayed in comparison with Tacrine as a reference drug. The retrieved protein (PDB: 2JEG) used for this purpose was improved by using import and preparation option of MVD software and missing bond order, hybridization state, angle and flexibility for achieving reliable potential binding site in receptor. The energy minimized ligands (synthesized compounds) were drawn with Chem Draw Ultra (2D and 3D). Discovery studio 3.5 [46] and MVD [47] software were used to perform molecular docking, energy profile of ligand-receptor interaction independently.

3.7. In vitro acetylcholinesterase inhibition activity

The *in vitro* inhibition of acetylcholinesterase (AChE) activity of the compounds (**8-10**) was carried out spectrophotometrically by Ellaman's coupled enzyme assay method using Tecrine as reference drug [48]. Electric ell AChE (Type-VI-S, EC 3.1.1.7) was used as the enzyme source, acetylthiocholine iodide as substrate and 5,5'-dithio-bis (2-nitrobenzoic) acid (DTNB) was used for the anti-cholinesterase activity determination. In this procedure, the assay solution was composed of 0.1mL of each sample (1 mg/mL in methanol), 0.02 mL of substrate (75 mM acetylthiocholine iodide in H_2O) and 0.1 mL of Ellman reagent (10 mM DTNB and 17.85 mM sodium bicarbonate in sodium phosphate buffer solution, pH 7.0). The reaction mixture was incubated for 15 minutes at 25 ºC, 25 µL of enzyme solution containing 0.28 U/mL (commercial acetylcholinesterase) was added to above mixture with 3.0 mL of sodium phosphate buffer and then incubated for 5 min at 25 ºC. The resulting solutions were placed in a spectrophotometer. For non-enzymatic reaction, the assays were carried out with a

blank containing all the components except acetylcholineaterase. The difference in absorbance at 412 nm for sample and control was taken as an inhibition rate (%). The percentage of enzyme inhibition was calculated using the following formula.

$$\% \text{ inhibition} = E - S/S \times 100$$

where E is the activity of enzyme without test sample and S is the activity of enzyme with test sample. Tacrine was used as a standard inhibitor. The experiments were done in triplicate and the results were expressed as an average values.

3.8. Antimicrobial activity

The *in vitro* antibacterial activity was carried out against nine bacterial strains (six gram positive and three gram negative). The strains so selected for the study are *Staphylococcus aureus* (ATCC 29213), *S. epidermedis* (MTCC 435), *Streptococcus mutans* (ATCC 25175), *S. pyrogenes* (MTCC 435), *S. viridans, Corynebacterium diphtheria,* (gram-positive bacterial strains) *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (MTCC 424) and *Proteus vulgaris* (MTCC 426) (gram-negative bacterial strains). These strains were screened for estimation of antibacterial activities of synthesized compounds. The antimicrobial activity was evaluated by Disc diffusion method [49,50]. Standard inoculums (1×10^7 to 2×10^7) c.f.u mL^{-1} (0.5 mcFarland standards) was introduced on to the surface of sterile agar plates and sterile glass spreader was used for even distribution of the inoculums. Every trial compound (1 mg) was dissolved in 100 µL DMSO to prepare stock solution and from stock solution diverse concentration 10, 20, 25, 50 and 100 µg/µL of every trial compound were prepared. After that the compounds of diverse concentration were poured over disk plate. The discs measuring 6 mm in diameter were prepared from Whatmann no. 1 filter paper and sterilized by dry heat at 140 ºC for 1 h. The sterile discs previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were also kept. Gentamicin was used as positive control. While the disc poured in DMSO was used

as negative control. The plates were inverted and incubated for 24 h at 37 ºC. The susceptibility was assessed on the basis of diameter of zone of inhibition against different strains of bacteria. Inhibition zones were measured and compared with standard drug. **Table 2-3**.

3.9. Antioxidant Studies

The acridone derivatives (**8-10**) were tested for their antioxidant property by 1,1-diphenylpicrylhydrazyl (DPPH) method [51-53]. In this procedure stock drug solution (1 mg/mL) was diluted to final concentration of 2, 4, 6, 8, 10 and 12 in methanol. Methanolic DPPH solution (1 mL, 0.3 mmol) was added to 3.0 mL of drug solution of different concentrations. The tube was kept at an ambient temperature for 30 min and the absorbance was measured at 517 nm by UV VIS spectrophotometer. The scavenging activity was calculated by following formula:

$$\% \text{ inhibition} = [A_{Control} - A_{Sample}) / A_{Control}] \times 100$$

where $A_{Control}$ is the absorbance of the L-ascorbic acid (Standard) and A_{Sample} is the absorbance of different compounds. The methanolic DPPH solution (1 mL, 0.3 mM) was used as control. The inhibitory concentration (IC_{50}) value represents the concentration required to exhibit 50% antioxidant activity. The IC_{50} values were calculated by the linear regression of plots where the abscissa represents the concentration of the compounds (µg/mL). Explicitly, IC_{50} is the average percentage of antioxidant activity. Results in the form of percent inhibition are tabulated in the **Table 4**. The experiments were done in triplicate.

4. Conclusions

We have successfully prepared acridone derivatives (**8-10**) starting from *o*-halobenzoic acid and substituted aniline derivatives by Ullmann condensation followed by cyclization of the ring. The simple method, mild conditions, high yields and particularly environmental friendliness make this procedure very attractive. All the compounds showed considerable antimicrobial activity against different strains of bacteria. The compounds also showed good antioxidant activity by the DPPH method.

Acknowledgments

The authors are thankful to the Chairman, Department of Chemistry, A.M.U., Aligarh for providing the necessary research facilities and the UGC is also gratefully acknowledged for providing the research fellowship to one of the co-authors (AA).

References

[1] J.H. Tan, Q.X. Zhang, Z.S. Huang, Y. Chen, X.D. Wang, L.Q. Gu, J.Y. Wu, *Eur. J. Med. Chem.* 41 (2006) 1041-1047.

[2] W.A. Denny, Curr. Med. Chem. 9 (2002) 1655-1665.

[3] L.K. Basco, S. Mitaku, A.L. Skaltsounis, N. Ravelomanantsoa, F. Tillequin, M. Koch, J.Le Bras, *Antimicrob. Agents Chemother.* 38 (1994) 1169-1171.

[4] G.S. Peter, B.T. John, Comprehensive medicinal chemistry: The rational design, mechanistic study & therapeutic applications of chemical compounds, Pergamon Press. 4 (2005) 4-5.

[5] G. Cholewinski, K. Dzierzbicka, A.M. Kołodziejczyk, *Pharmacol. Rep.* 63 (2011) 305-336.

[6] J.X. Kelly, M.J. Smilkstein, R. Brun, S. Wittlin, R.A. Cooper, K.D. Lane, A. Janowsky, R.A. Johnson, R.A. Codean, R. Winter, D.J. Hinrichs, M.K. Riscoe, *Nature* 14 (2009) 270-273.

[7] C.T. Lowden, K.T. Bastow, *Antiviral Res.* 59 (2003) 143-154.

[8] C.S. Sepulveda, M.L. Fascio, C.C. Garcia, N.B. DAccorso, E.B. Damonte, *Curr. Med. Chem.* 20 (2013) 2402-2414.

[9] A, Boumendjel, S. Macalou, A. Ahmed-Belkacem, M. Blanc, A.Di Pietro, *Bioorg. Med. Chem.* 15 (2007) 2892-2897.

[10] Y.C. Mayur, G.J. Zaheeruddin Peter, C. Lemos, L. Kathmann, V.V.S. Rajendra Prasad, *Arch. Pharm. Chem. Life Sci.* 342 (2009) 640-650.

[11] P. Singh, J. Kaur, B. Yadav, S.S. Komath, *Bioorg. Med. Chem.* 18 (2010) 4212-4223.

[12] T.R. Krugh, *Curr. Opin. Struct. Biol.* 4 (1994) 351-364.

[13] S. Khan, S.A.A. Nami, K.S. Siddiqi, *J. Organomet. Chem.* 693 (2008) 1049-1057.

[14] K. Thimmaiah, A.G. Ugarkar, E.F. Martis, M.S. Shaikh, E.C. Coutinho, M.C. Yergeri, *Nucleosides Nucleotides Nucleic Acids*, 34 (2015) 309-331.

[15] R. Hegde, P. Thimmaiah, M.C. Yerigeri, G. Krishnegowda, K.N. Thimmaiah, P.J. Houghton, *Eur. J. Med. Chem.* 39 (2004) 161-177.

[16] A. Mukherjee, W.D. Sasikala, *Adv. Protein Chem. Struct. Biol.* 92 (2013) 1-62.

[17] W.A. Denny, *Med. Chem. Rev.* 1 (2004) 257-266.

[18] P. Belmont, I. Dorange, *Expert Opin. Ther. Patents* 18 (2008) 1211-1224.

[19] P.A. Datar, *J. Pharma. Anal.* 5 (2015) 213- 222.

[20] R. Kumar, M. Kaur, M. Kumari, *Pharma. Drug Res.* 69 (2012) 3-9.

[21] T. Faller, K. Hutton, G. Okafo, A. Gribble, P. Camilleri, D.F. Games, *Chem. Commun.* (1997) 1529-1530.

[22] A. Szymanska, K. Wegner, L. Lankiewitz, *Helv. Chim. Acta* 86 (2003) 3326-3331.

[23] N. Bahr, E. Tierney, J.L. Reymond, *Tetrahedron Lett.* 38 (1997) 1489-1492.

[24] J.L. Reymond, T. Koch, J. Schroder, E. Tierney, *Proc. Natl. Acad. Sci. U.S.A.* 93 (1996) 4251-4256.

[25] A. Bernthsen, *Ann* 1 (1878) 192.

[26] H. Meyer, A. Hofmann, *Monatsh fur Chimie* 37(1916) 681-722.

[27] G. Koller, E. Krakauer, *Monatsh fur Chimie* 50 (1928) 51-54.

[28] M.V. Gorelik, S.P. Titova, *Russ. Chem. Bull.* 56 (2007) 1679- 1680.

[29] J. Zhao, R.C. Larock, *J. Org. Chem.* 72 (2007) 583-588.

[30] R. Nishio, S. Wessely, M. Sugiura, S. Kobayashi, *J. Comb. Chem.* 8 (2006) 459-461.

[31] H.M.T.B Herath, K. Muller, H.V.K Diyabalanage, *J. Heterocycl. Chem.* 41 (2004) 23-28.

[32] L. Xuarez, R.F. Pellon, N. D'Accorso, V. Montesano, M. Fascio, *Heterocycles* 63 (2004) 23-28.

[33] J.P. Galy, J.P. Hanoun, V. Pique, N. Jagerovic, J. Elguero, *J. Heterocycl. Chem.* 34 (1997) 1781-1787.

[34] I. Tanasescu, M. Suciu, Sur la Condensation des o-Nitrobenzaldehydes Avec l'Aniline (III) (1). Comportement Photophimique des Anthraniles et Triphenylmethanes Obtenus, *Bull. Soc. Chim. France* 4 (1937) 245-255.

[35] A.S. Vincek, R.G. Booth, *Tetrahedron Lett.* 38 (1997) 5107-5109.

[36] T.K. Yazicilar, O. Andac, Y. Bekdemir, H. Kutuk, V.T. Yilmaz, W.T.A. Harrison, *Acta Crystallographica Section C58* (2002) 21-22.

[37] International Tables for X-Ray Crystallography, Kynoch Press, Birmingham, England, vol. III (1952).

[38] SAINT, Version 6.02, Bruker AXS, Madison, WI, (1999).

[39] XPREP, Version 5.1, Siemens Industrial Automation Inc., Madison, WI, (1995).

[40] G. M. Sheldrick, SHELXL-97: Program for Crystal Structure Refinement, University of Gottingen, Gottingen, Germany, (1997).

[41] R. Thomsen, M.H. Christensen, MolDock: A new technique for high-accuracy molecular docking. *J. Med. Chem.* 11 (2006) 3315-3321.

[42] D. Schneidman-Duhovny, Y. Inbar, R. Nussinov, H.J. Wolfson, PatchDock and SymmDock: servers for rigid and symmetric docking. *Nucl. Acids. Res.* (2005) 33 363-367.

[43] Accelrys Software Inc. Discovery studio modeling environment. Release (3.1.2011).

[44] J.M. Yang, C.C. Chen, GEMDOCK: A generic evolutionary method for molecular docking, Proteins 55 (2004) 288-304.

[45] A. Panjkovich, X. Daura, *Bioinformatics* 30 (2014) 1314-1315.

[46] Accelrys software inc., Discovery studio modeling environment. Release 3.1. (2011).

[47] R. Thomsen, M.H. Christensen, *J. Med. Chem.* 48 (2006) 3315-3321.

[48] G.L. Ellman, K.D. Courtney, V.J. Andres, R.M. Featherstone, *Biochem. Pharmacol.* 7 (1961) 88- 95.

[49] A.H. Collins, Microbiological Methods; Butterworth: London, 4th Ed. 11, (1976) 24.

[50] R. Cruickshank, J.P. Duguid, B.P Marmion, R.H. Swain, Medicinal microbiology, 12th ed., Churchill Livingstone, London, vol. (II) (1975) 196-202.

[51] K. Kato, S. Terao, N. Shimamoto, M. Hirata, *J. Med.Chem.* 31 (1988) 793-798.

[52] M.S. Blois, *Nature* 181 (1958) 1199-1200.

[53] S.A.A. Nami, M. Alam, A. Husain, M. Parveen, *Spectrochim. Acta Part A* 96 (2012) 729-735.

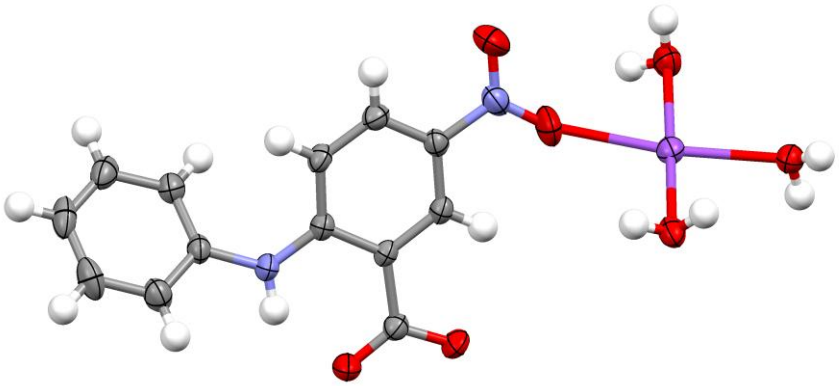


Fig.1. X-ray crystallographic structure of compound **(4)**.

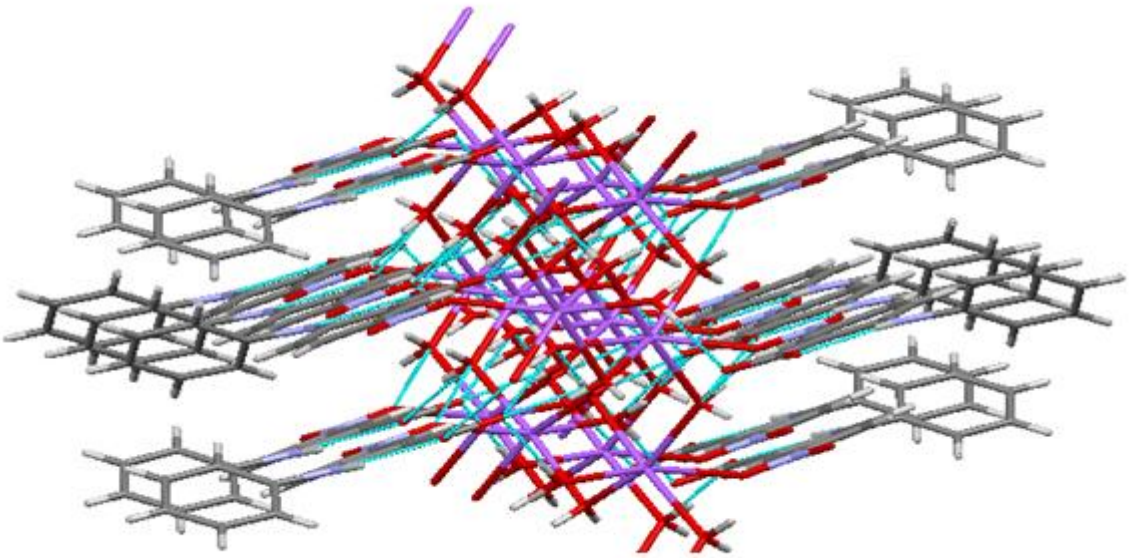


Fig.2. Hydrogen bonding network in compound (**4**) with the H-bond depicted as dashed lines.

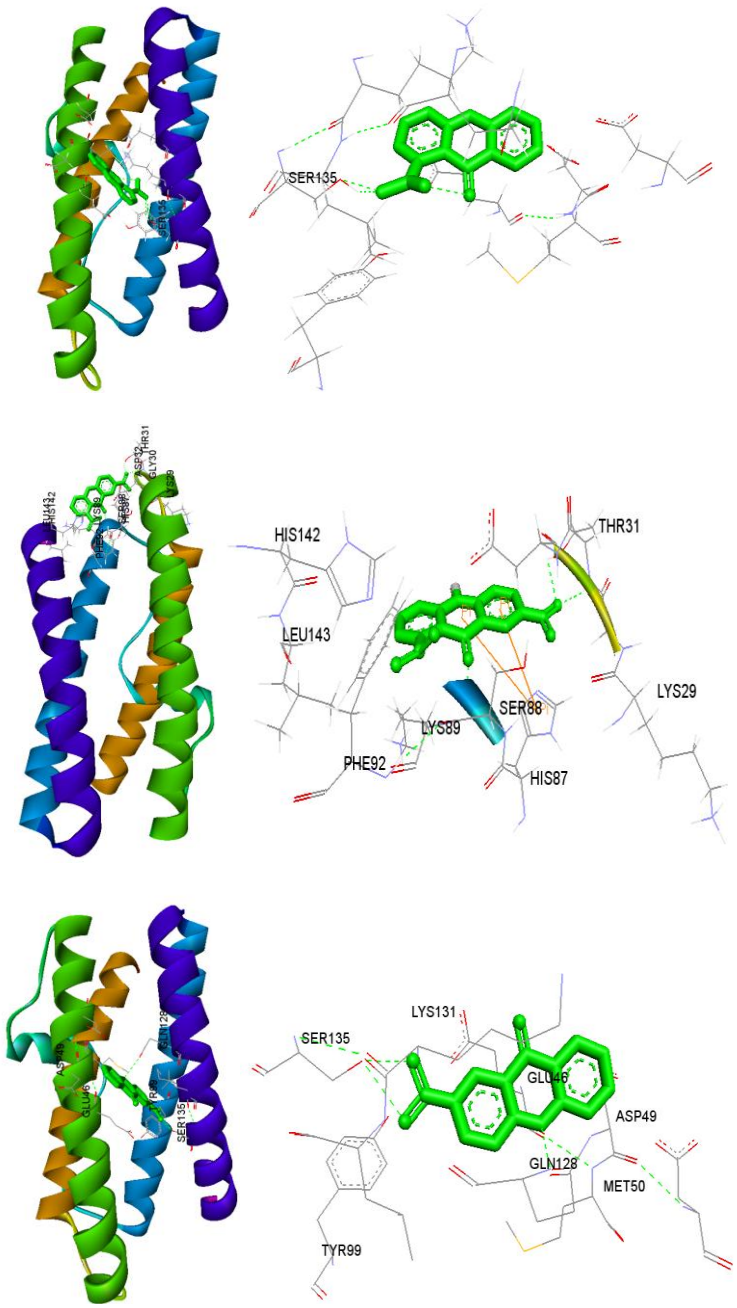
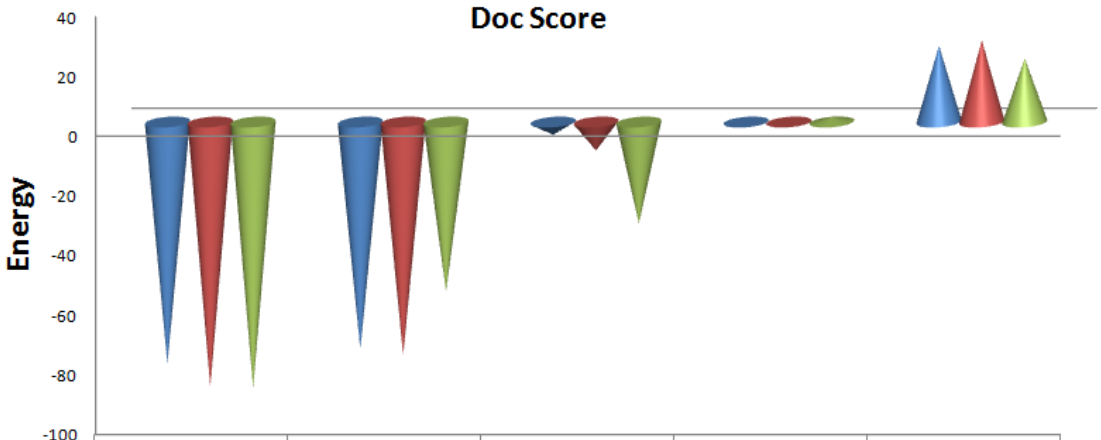
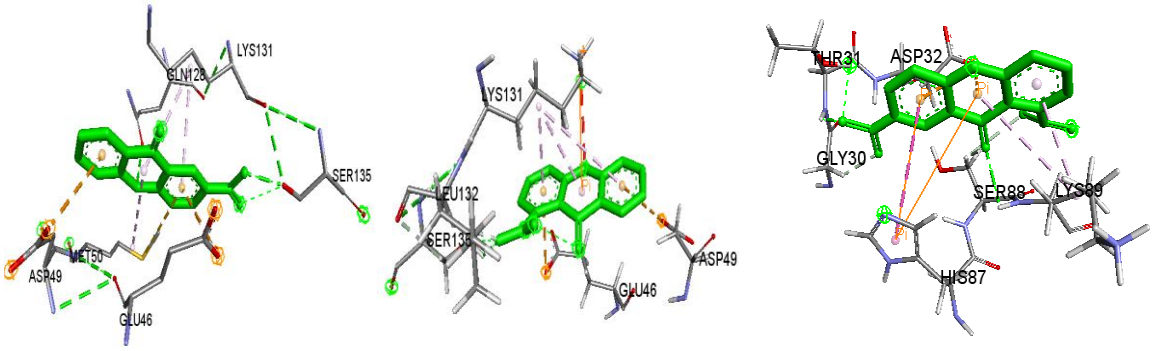


Fig.3. The strong binding affinity of compounds (**8**-**10**) with AChE explained on the basis of hydrogen bonding/secondary interactions as well as orientation and electronic features of the substituents towards the active site of the target enzyme.



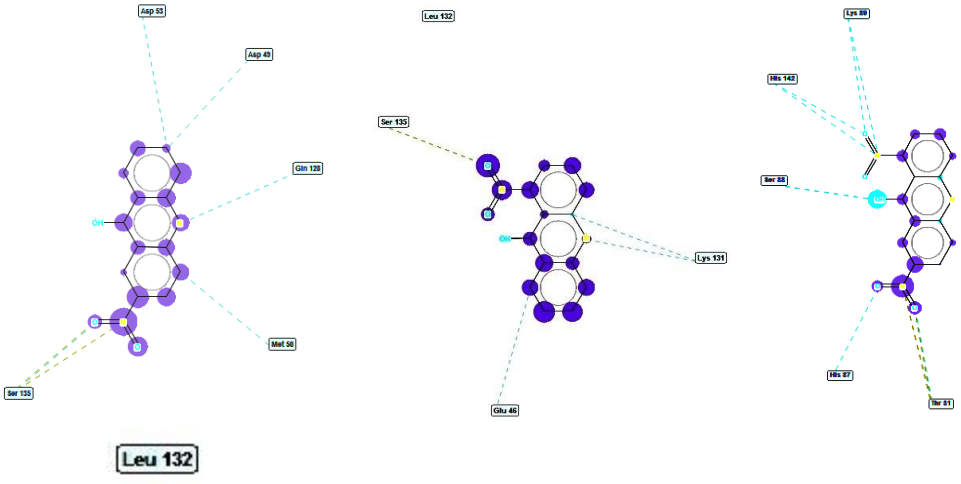
	TotalEnergy	VDW	HBond	Elec	AverConPair
Series1	-81.0462	-76.0528	-4.25602	-0.737396	25.6111
Series2	-88.6661	-78.1459	-9.52593	-0.994295	27.4444
Series3	-89.1203	-56.8182	-33.9665	1.66435	21.381

Fig.4. Estimated binding affinities of synthesized compounds (**8-10**) based on docked poses within active site of target enzyme (PDB: 4ey4)



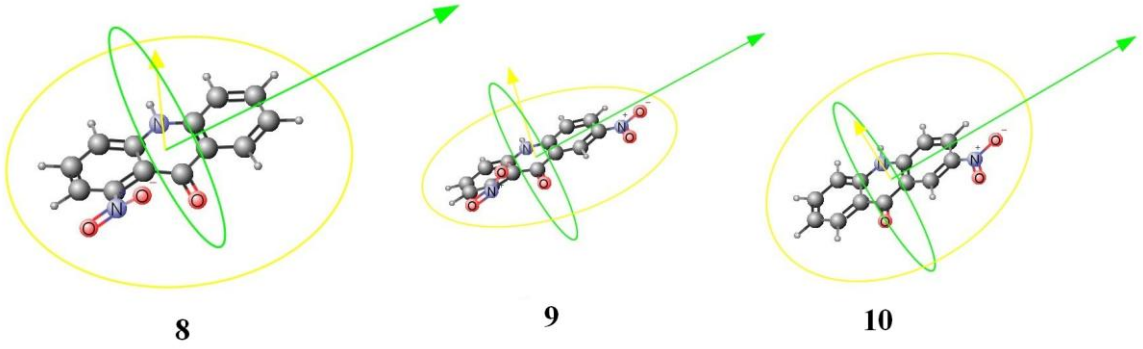
8 **9** **10**

Fig.5. π-π interaction of compounds (**8**-**10**) with amino acid residues of the target enzyme.



8 **9** **10**

Fig.6. Ligand map of the synthesized compounds showing interactions between the docked molecules and active site region of the protein.



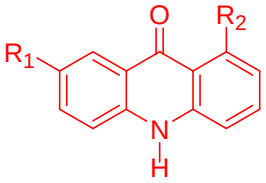
8 **9** **10**

Fig. 7. Geometrical structure of compounds **8**-**10** showing Van der Waals volume and length perpendicular to the maximum and minimum area. Green, yellow circle represents the radius of the circumscribed circle of the minimal and maximal projection of the current conformer, while, green and yellow arrow for min z and max z length of the conformer perpendicular to its minimal and its maximal projection area, respectively.

Table 1 Crystallographic data and structure refinement of compound (**4**)

Compound	**4**
Empirical formula	$C_{13}H_{15}N_2NaO_7$
Formula wt.	334.26
Temperature	293 (2)
Wavelength	0. 71073
Crystal system	Monoclinic
Space group	*P21/c*
a, Å	20.0793(6)
b, Å	10.4674(3)
c, Å	7.2945(2)
α (°)	90
β (°)	99.018(2)
γ (°)	90
U, Å^3	1514.19(8)
Z	4
ρ calc Mg/m^3	1.466
μ, mm-^{1}	0.143
F(000)	696
Refl. Collected	3363
Independent refl.	2870
GOF	1.122
Final R indices	^{a}R1= 0.0359
[I>2σ(*I*)]	$^b{}_w$R2= 0.1011(3359)
R indices	R1= 0.0348(2870)
(all data)	$_w$R2= 0.1123

Table 2 Quantitative estimation of acetylcholinesterase inhibition activity of compounds (**8-10**) by modified Ellaman's coupled enzyme assay method using tacrine as reference (**n=3**).



Compound	Nature of Substituents	IC_{50} (µM)[a] ± SEM[b] for *h*AChE[c] inhibition
10	R_1= NO_2, R_2= NO_2	0.22±0.01
9	R_1= H, R_2= NO_2	0.31±0.01
8	R_1= NO_2, R= H	0.45±0.01
Standard (Tacrine)		0.20±0.01

[a]**IC_{50}** values represent the concentration of inhibitor required to decrease enzyme activity by 50%
[b]**SEM** = standard error of the mean
***h*AChE =** Human recombinant AChE from human serum

Table 3 Geometrical descriptors data of synthesized compounds (**8-10**)

Compound / Descriptors	**8**	**9**	**10**
Minimal projection area	27.06	28.69	30.19
Maximal projection area	75.06	73.01	81.02
Minimal projection radius	4.38	4.23	4.36
Maximal projection radius	6.48	6.02	6.49
van der Waals Volume	189.57	189.80	212
Length perpendicular to the max area	4.32	4.96	5.15
Length perpendicular to the min area	12.94	11.54	12.98

Table 4 Estimation of zone of inhibition of compounds (8-10) against different Gram positive bacterial stains

	Zone of Inhibition (mm) Bacterial strains (Gram positive)					
Compound	*S.aureus*	*S.mutans*	*C.diphtheria*	*S.epidermidis*	*S.viridans*	*S.pyrogenes*
8	14	-	13	12	-	8
9	15	10	-	16	-	10
10	18	12	12	14	14	-
Ampicillin	16	15	18	18	15	11
DMSO	-	-	-	-	-	-

Standard drug = Ampicillin
Negative control = Dimethyl sulphoxide (DMSO)

Table 5 Estimation of zones of inhibition (mm) of compounds (8-10) against different Gram negative bacterial stains

Compound	Zone of Inhibition (mm) Bacterial strains (Gram negative)		
	Escherichia coli	*P. aeruginosa*	*Proteus vulgaris*
8	7	8	14
9	11	7	16
10	9	10	-
Gentamicin	28	18	18
DMSO	-	-	-

Standard drug = Gentamicin

Negative control = Dimethyl sulphoxide (DMSO)

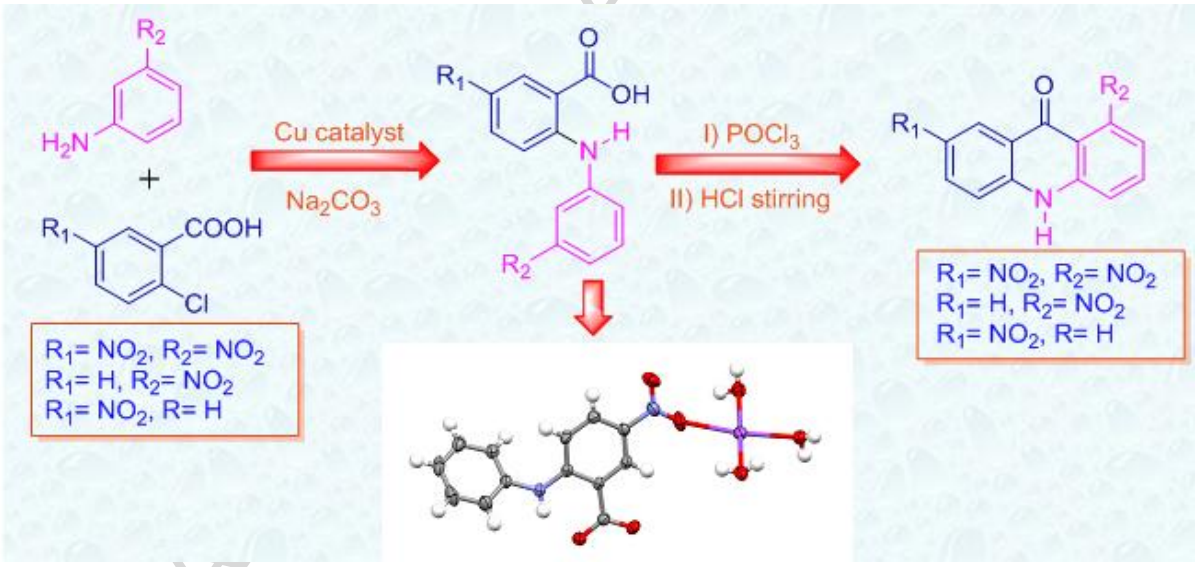
Table 6 DPPH scavenging activity data of compound (**8-10**) (n=3)

S. No	Compounds	Absorbance	Absorbance at 517 nm				IC_{50}
			25µ/ml	25µ/ml	25µ/ml	25µ/ml	
1	Control	0.751± 0.03	0.751±0.03	0.751±0.03	0.751±0.03	0.751± 0.03	
2	**8**	Abs	0.380±0.07	0.345±0.08	0.246±0.04	0.171±0.04	31.58
		(AA%)	49.40	54.06	67.24	77.23	
3	**9**	Abs	0.407±0.04	0.320±0.03	0.252±0.04	0.169±0.04	34.25
		(AA%)	45.80	57.39	66.40	77.49	
4	**10**	Abs	0.371±0.02	0.345±0.04	0.276±0.03	0.220±0.05	27.80
		(AA%)	50.59	54.06	63.24	70.70	
5	Ascorbic acid	Abs	0.414±0.03	0.279±0.04	0.190±0.03	0.101±0.03	
		(AA%)	44.8	62.8	74.7	86.5	31.11

Graphical abstract (pictogram)

Potent Acetylcholinesterase Inhibitors: Synthesis, Biological Assay and Docking Study of Nitro Acridone Derivatives

Mehtab Parveen*[,1], Afroz Aslam[1], Shahab A. A. Nami[2], Ali Mohammed Malla[1], Mahboob Alam[3], Dong-Ung Lee[3], Sumbul Rehman[4], P. S. Pereira Silva[5], M. Ramos Silva[5]
[1]Department of Chemistry, Aligarh Muslim University, Aligarh 202002, India
[2]Department of Kulliyat, Faculty of Unani Medicine, Aligarh Muslim University, Aligarh 202002, India
[3]Division of Bioscience, Dongguk University, Gyeongju 780-714, Republic of Korea
[4]Department of Ilmul Advia, Faculty of Unani Medicine, Aligarh Muslim University, Aligarh 202002, India
[5]CEMDRX, Physics Department, University of Coimbra, P-3004-516 Coimbra, Portugal.



Synthesis of Acridone Derivatives

Research Highlights

- Synthesis of nitro acridone derivatives.
- Crystal structure of 5-nitro-(2-phenyl amino) benzoic acid.
- Acetylcholinesterase (AChE), antimicrobial inhibitory and antioxidant activities.
- Docking studies