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SYNTHESIS, ANTIMICROBIAL EVALUATION AND MOLECULAR DOCKING OF SOME PYRIDINE, PYRAN, PYRAZOLINE AND/OR ISOXAZOLINE-9-(*P*-SUBSTITUTEDANILINO)-ACRIDINE DERIVATIVES

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ABSTRACT

A new series of nitrogen and/or oxygen heterocyclic- 9-(*p*-substituted anilino) acridines were synthesized for the purpose of antimicrobial evaluation. These heterocycles include pyridinecarbonitrile, iminopyridine, pyrans, acridine-chalcones, 4,5-dihydropyrazoline and/ or isoxazoline ring systems. Some representative examples of these compounds compared with other known derivatives, showed considerable antimicrobial activity against G +ve, G -ve bacteria and fungi. Molecular docking studies of the affinity of binding of these compounds in the DNA gate of the bacterial gyrase enzyme were carried out.

KEY WORDS: Acridines; Pyridines; Pyran; Pyrazolines; Isoxazolines, Antimicrobial, Molecular docking.

INTRODUCTION

Acridine derivatives are widely used in medicine as pharmaceutical agents [1-6]. Most of the acridine derivatives are focused on the 9-substituted amino or substituted anilino acridine scaffold, paying more attention on the substituents effects in the acridine and aniline rings on the DNA binding ability and cytotoxicity [7-12]. Acridines are firstly used as antibacterial and antimalarial agents with planar aromatic structures that are capable to intercalate into DNA base pairs [5,6,13]. Also acridine compounds have a long history as anticancer [1,2], anti-oxidant [3] and anti-inflammatory [4]. Their potentials as antitumor drugs have been previously examined. For example, *N*,*N*-dimethyl-*N*'-(l-nitro-9-acridinyl)-l,3-propanediamine (Fig. **1** Nitracrine) showed potent antineoplastic activity [14,15]. Also, l-[(6-chloro-2-(methoxy-9-acridinyl)-amino]-3-diethylamino-2-propanol dihydrochloride (Fig. 1 Acranil) is a known antiviral agent. It exhibits interferon induction and radio protective activities [16].It is generally assumed that most of the biological activity of these compounds is connected with their well-established DNA intercalation due to its flat structure. Each intercalating drug binds strongly to particular base pairs as result of several interactions, ranging from van der Waals forces to the formation of hydrogen bonds with adjacent nucleobases [17-19]. Their binding to DNA is dependent on a number of structural factors, particularly the substituents on both the acridine nucleus and the 9- amino or anilino function groups [20-24]. 9-(pyridin-2'-yl)-aminoacridines were prepared and analysed for their ability to change the thermal denaturation temperature of genomic calf thymus DNA [25]. On the other hand, several pyridones, pyrans, pyrazolines and/or isoxazoline derivatives were found to possess considerable antimicrobial activity [26-31].

The aim of the present work is to synthesize a new series of acridine derivatives incorporated at its 9-position

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into the above mentioned heterocyclic systems through a *p*-iminophenyl moiety. The presence of this binary system in one molecule may results in a dual mode of biological action which in turn may increase the antimicrobial activity.

Chemistry

The chemical modification of 9-anilinoacridines, such as the introduction of different substitutions or heterocyclic ring systems were allowed expansion of SAR studies to afford new insight into molecular interaction at receptor level in the parasite cell. Recently, it is well established that slight structural modification on the substitution at the 9- position of acridine ring may bring various biological activities [25,32].

Also, it is well known that chalcones are considered as excellent starting materials for the synthesis of 2(1*H*) pyridones in a reaction where Aldol condensation product reacts with ethyl cyano acetate in the presence of ammonium acetate [22]. This procedure is time consuming and experiences some difficulties especially with propenones bearing heterocyclic moieties. So, in the present work, a method for one-step synthesis of some new cyanopyridones, cyanoiminopyridines and aminopyrans incorporated to acridine through a *p*-iminophenyl group at the 9-position of the acridine moiety is reported [26] by which good yields of the products were obtained.

The starting material of choice was 9-(p-acetylanilino)-acridine (**1**) [33] which allowed to react with ethyl cyano acetate and 3,4-dihydroxybanzaldehyde in the presence of ammonium acetate to afford the corresponding 3-cyano-2(1H)pyridone derivative 2e as an example of the one step Michael condensation reaction according to a reported method [26] (Scheme 1).

The IR spectra of compounds 2a-e showed bands due to NH (3350), typical 2(1H) pyridone NH (3160), -CN (2200) cm-1 and C=O (1660). ^{1}H NMR spectrum of the new compound 2e (DMSO-d_6) showed signals at δ 7.2-8.2 ppm (m, 16H aromatic), δ 8.3 (s, 1H amide) and at δ 8.7 (s, 1H, NH). MS spectrum of **2e** showed a peak at m/z 194.1 ($C_{13}H_9N_2$) (100% base peak) and M^+ at m/z 496 $C_{31}H_{20}N_4O_3$ (9%).

In the same manner, reaction of 1 with malononitrile and the appropriate aldehyde under the same reaction conditions, afforded the corresponding 2 (1H) iminopyridines **3a-c**. [26]. The IR spectrum of **3b** showed bands attributable to 2-NH_2-pyridine at 3480, 3290 cm^{-1}, NH at 3220 cm^{-1}, 2180 (CN) and at 1600 cm^{-1} (C=C). ^{1}H NMR of the new compound **3b** (DMSO-d_6) showed: δ 2**.**5 ppm (s, 3H, CH3), δ 7.0-8.4 ppm (m, 17H aromatic), δ 8.8, 9.0 (s, 1H, NH and br s, 2H, NH2) and its MS showed molecular ion peak of C32H23N5 M+ at m/z 477 (5%) However, the reaction of 1 with malononitrile and an aldehyde in presence of piperidine afforded the corresponding 2-aminopyrans **4a,b** respectively (Scheme 1). The IR spectrum of **4** showed bands due to NH_2 at 3450, 3300 cm^{-1}, CN at 2115 cm^{-1} and C=C at 1605 cm^{-1}. The MS of 4b showed the molecular ion peak $C_{32}H_{23}N_4O$ M^+ at m/z 479 (10%).

On the other hand, since chalcones (α,β-unsaturated ketones) are useful starting materials for the synthesis of may heterocycles such as pyrazoles, isoxazoles and pyridines, it was of interest to synthesize a new series of 9-(4-substilutcd anilino) acridines incorporated at the 4-position of aniline with these mentioned heterocycles for biological evaluation. Claisen-Schmidt condensation of the acetyl derivative **1** with the appropriate aldehyde in 5-10% ethanolic sodium hydroxide solution gave a poor yield of the chalcones **5** in addition to many other by-products. Also, when the reaction of **1** with aromatic aldehydes was carried out in acetic acid in presence of sodium acetate, low yields of **5** were obtained.

Another attempt was carried out by using the method described by Scholtz [34,35] in which the α,β-unsaturated ketone was prepared firstly from an aldehyde and *p*-aminoacetophenone then reacting the obtained 4-substituted cinnamoylaniline HCl salts with the required substrate , namely 9-chloroacridine. Good yields (55-70%) of the desired chalcones namely, acridine-9-[4-(3-substituted arylacryloyl)phenyl]-amines **(5a-d)** were obtained (Scheme 2). IR of **5a,b** showed band at 3400 (NH), 1668 (C=O). 1H NMR of **5b** (DMSO-d_6) showed bands at δ 3.84 ppm (s, 3H, OCH_3), δ 7 (2H, dd, CH=CHCO) and at δ 7.3-8.3 (m, 16H aromatic). The MS of the new chalcone derivative 5d displayed the molecular ion peak of $C_{26}H_{18}N_2O_2$ at M^+ m/z 390 (10%).

The α,β-unsaturated ketones **5** were allowed to react with 98% hydrazine hydrate in ethanol to give the corresponding pyrazolines **6a,b**. When the reaction was carried out in acetic acid, the *N*-acetylpyrazoline derivative **6c** was obtained. Reaction of compounds **5a, 5d** with hydroxylamine hydrochloride in ethanolic sodium hydroxide solution afforded the corresponding isoxazolines **7a,b** respectively. Structures of the pyrazolines **6** and the isoxazolines **7** were inferred from the chemical analyses and spectral data.IR spectrum of 6c showed absorption bands at 3280 cm-1 (NH), 1668 cm-1 (C=O, acetyl), and at 1600 cm-1 (C=C). 1H NMR of 6c (DMSO-d_6) showed bands at δ 2.4 (s, 311, acetyl), 3.4, 4.9 (dd, dd, 1H, 1H, -CH2 of pyrazoline), 4.2 (dd, 1H, CH of pyrazoline), 7.2-8.3 (m, 17H aromatic) and at 9.3 (s, 1H, NH). MS of **7a** showed peaks of M^+ $C_{28}H_{21}N_3O$ at *m/z* 415.2 (1%) and $C_{13}H_9N_2$ at *m/z* 194 (100%), and its IR spectrum showed bands at 3310 (NH), 3075 (CH aromatic), 2940 (CH-aliphatic), and 1600 (C=C). Reaction of **5a** with ethyl cyano acetate in the presence of ammonium, acetate at 150°C, afforded the same l,6-dihydro-3-cyano-2(lH) pyridone derivative **2a** that previously obtained by the one step reaction (Scheme 1) as indicated by its similar melting and mixed melting point (195-7°C) but in 30% yield compared to the one pot reaction (66% yield) (Scheme 2).

EXPERIMENTAL

Melting points are uncorrected and were taken an electrothermal capillary melting point apparatus the IR spectra were recorded (KBr disks) on Perkin Elmer model 137 infracord spectraphotometer the "H NMR spectra were measured in DMSO-d_6 on Joel Ex-27 MHz spectrometer. The mass spectra were recorded on GCMS-Q 1000 Ex Schimadzu gas chromatography MS apparatus Microanalyses were carried out at the microanalytical unit, Faculty of Science, Cairo University and National Research Centre, Egypt. Carbon, Hydrogen and Nitrogen analyses of the new compounds were found to be identical with the calculated molecular weights within a range or error between (+ or - 0.4%).

6-[4-(Acridin-9-ylamino)phenyl]-2-oxo-4-(3,4-dihydroxyphenyl)-l,2-dihydropyridine-3-carbonitrile (2e) General method [26].

A mixture of the acetyl compound **1**(24) (3.129, 0.01 mole), ethylcyanoacetate (1.13g, 0.01 mol), 3,4-dihydroxybenzaldehyde (0.01 mol) and ammonium acetate (4.62g, 0.06 mol) in 50 ml n-butanol was heated under reflux for 6 hrs. A crystalline solid was obtained, filtered off, washed with water then with cold ethanol and crystallized from ethanol to give compound **2**e, m.p. 200 oC(ethanol) in 65% yield, Anal, C31H20N4O3, Calcd. C 75.00, H 4.03, N 11.29, Found: C 74.60, H 3.85, N 11.41%. Melting points of compounds 2a-d are 197,255,220 and 210 ^{o}C respectively [26].

6-[4-(Acridin-9-ylamino)phenyl]-2-imino-4-aryl-1,2-**dihydropyridine-3-carbonitriles (3a-c):General method [26].**

A mixture of **1** (3.12 gm, 0.01 mol) malononitrile (0.01 mol), p-tolualdehyde (0.01 mol) and ammonium acetate (0.985g; 0.0128 mol) in n-butanol was refluxed for 3 hours. The solid formed was filtered, washed with H_2O then petroleum ether, dried and crystallized from ethanol to give compound 3b, m.p 198-200 ^{o}C (ethanol), Anal, $C_{32}H_{23}N_5$, Calcd C, 80.50, H 4.82, N, 14,67, Found, C, 80.72, H, 5.15, N, 14.90%. Melting points of compounds 3a and 3c are 270 and 278 ^{o}C respectively [26].

6-[4-(Acridin-9-ylamino)phenyl]-2-amino-4-aryl-4H-pyran-3-carbonilriles (4a,b):

A mixture of **1** (3.l2g; 0.01 mol), malononitrile (0.65g, 0.01 mol), an appropriate aldehyde (0.01 mol) and few drops of piperidine in n-butanol (50 ml) was refluxed for 5 hours. The solid formed was filtered, washed with H_2O then cold ethanol and crystallized from dioxane to give compounds **4a,b**, m.p. 280, 292 ^{o}C respectively

l-[p-(Acridin-9-ylamino)phenyl]-3-aryl-propen-l-ones (5a-d):

General method:

A mixture of 9-chloroacridine [33] (2g, 0.01 mol) and the appropriate substituted cinnamoyl aniline hydrochlorides (0.01 mol), prepared by the method of Scholtz [34], in ethanol (20 ml) and few drops piperidine was refluxed for 4h. The reaction mixture was cooled and the precipitated material was filtered off, crystallized from the proper solvent to give compounds **5a-d**. 1-[p-(Acridin-9-ylamino)phenyl]-3-(furan-2-yl)-propen-1-one (**5d**), m.p. 260 ^{o}C (AcOEt), Analysis, C26H18N2O2, (390) Calcd. C, 80.02, H, 4.61, Found, C, 79.55, H, 4.90%

9-[p-(5-Aryl-4,5-dihydro-lH-pyrazolin-3-yl) anilino] acridines (6a-c).

General method:

A mixture of the chalcone derivatives **5a,b** (0.015 mol) and 98% hydrazine hydrate (0.5g, 0.015 mol) in absolute ethanol (10 ml) was refluxed for 3h. The precipitated product was filtered off and crystallized from the proper solvent to give compounds **6a,b** respectively. When a mixture of compound **5a** (6g, 0.015 mol) and 98% hydrazine hydrate (0.5g, 0.015 mol) in glacial acetic acid (5 ml) was refluxed for 4h a precipitated product of N-acetylpyrazoline derivative **6c** was filtered off and crystallized from acetone.

Compound 6a, m.p. 212-213 oC (ethanol), Analysis, C28H22N4, (414) , Calcd., C, 81.15, H, 5.31, N, 13.52, Found, 80.90, H, 5.70, N, 14.02% **Compound 6b**, m.p. 196-7 ^{o}C (ethanol), Analysis, C29H24N4O (444), Calcd., C, 74.17, H, 5.40, N,12.61, Found, C,73.90, H, 5.70, N, 12.40%. **Compound 6c**, m.p. 186-7 ^{o}C (acetone), Analysis, C30H24N4O (456), Calcd., C, 78.84, H, 5.26, N12.28, Found, C, 78.80, H, 5.46, N, 12.52%.

9-[p-(5-Aryl-4,5-dihydro-lH-isoxazolin-3-yl) anilino] acridines (7a,b).

A mixture of compound **5a,d** (0.015 mol) hydroxylamine hydrochloride (l g, 0.015 mol) and sodium hydroxide (0.lg) in absolute ethanol (5 ml) was refluxed for 8 hours, and then poured onto ice water. The obtained precipitate was filtered off and crystallized from ethanol to give compounds **7a,b**. respectively. **Compound 7a:** m.p. 214 ^{o}C (ethanol), Analysis, C28H21N3O, (415), Calcd. C, 80.69, H, 5.06, N, 10.12, Found, C 81.08, H, 4.95, N, 10.10.41%. Compound 7**b**: m.p. 200-201 ^{o}C (ethanol), Analysis, C26H19N3O3 (405), Calcd. C, 77.03, H, 4.69, N, 10.37, Found, C, 76.85, H, 4.88, N, 10.50%

6-[4-(Acridin-9-ylamino)phenyl]-2-oxo-4-phenyl-],2-dihydro-pyridine-3-carbonitrile (2a):

Method 2: From the chalcone 5a

A mixture of compound **5a** (0.4g, 0.001 mol), ethylcyanoacetate (0.226g, 0.002 mol) and ammonium acetate (0.308g, 0.004 mol) in n-butanol (5 ml) was heated under reflux for 5h. A crystalline solid was separated, filtered and crystallized from ethanol to give compound 3a in 30% yield. The obtained product was found to be

identical with that obtained by the one pot reaction from compound **1** (Scheme 1). M.p. 196 °C mixed m.p. 194°C.

Biology

The selected compounds were tested against *Escherichia Coli*, *Pseudomonas Aeruginosa*, *Staphylococcus Aureus*, *Sarcina Lutea*, *Bacillus Subtilis*, *Mycobacterium Phlei*, and *Candida Albicans* and *Aspergillus Niger*. The antimicrobial activity of the tested compounds was determined in side-by-side with Nalidixic acid [36] as antibacterial reference drug or Clotrimazol [37] as antifungal reference drug.

MATERIALS

All microorganisms used were obtained from the culture collection of the department of Microbiology and Immunology, Faculty of Pharmacy, Helwan University. The compounds were tested against *Escherichia Coli*, *Pseudomonas Aeruginosa*, and *Staphylococcus Aureus* in nutrient both, pH 7.0 and against *Bacillus Subtilis* in lacto brain heart infusion broth and against *Sarcin Lutea*, and *Candida Albicans* in broth containing 1% neopeptone, 2% dextrose with pH 5.7. Media for disc sensitivity tests were nutrient agar and Muller-Hinton agar (MHA) purchased from Difco. The disc diameter was 5mm. Non sterile powder of tested compounds were dissolved in DMSO to yield 5,000 ug/ml passed through 0.0002mm membrane filters (Millipore corp. Bedford, Mass).

Disc diffusion test

20 ml of Muller-Hinton agar (MHA) at 55°C, inoculated with 1 ml of the-microbial culture (10^6 CFU/ml), was poured in sterile Petri dish and left to solidify. A sterile filter paper disc impregnated with solution of the compound under testing (0.1 mg/ ml in DMF) was placed on the surface of agar, and the plate was incubated over night at 37°C. The diameter of the zone of inhibition was measured and compared with the standard zone produced by Nalidixic acid only for antibacterial evaluation and with Clotrimazol for antifungal evaluation [38].

Sequence alignment

Alignment was done for the fasta sequence for all Bacillus subtilis (Uniprot code = P39814) and E-coli (uniprot code = P06612) and Staph. aerus (uniprot code = Q2FHI8) using clustal omega program

Molecular docking

Docking was done using molecular operating environment [39,40].

RESULT AND DISCUSSION

Table 1 show:

- Compounds **2a-e** and **6a,d** showed high significant activity against *Bacillus Subtilis*, compound **1** is less active, and compound **2b** is moderately active against *Staphylococcus Aureus*.
- Compound **1** only showed moderate activity against *Eschericia C*oli, compared with Nalidixic acid.
- Compound **2e** showed moderate activity against *Mycobacterium Pheli*.
- Compound **6a** showed moderate activity against *Aspergillus Niger* (fungus) compared with the highly active Clotrimazole. 5- All compounds are not active against *Sarcina Lutea*, *Candida Albicans*, *Pseudomonas Aergmosa*.

Sequence alignment:

The antimicrobial activity of the compounds showed that all compounds were active on the *Bacillus Subtilis* and some of them were active against both *E. Coli* and *S. Aureus*. In order to interpret this sequence alignment between the gyrase enzyme of *Bacillus Subtilis* against both *E. Coli* and *S. Aureus* was performed which revealed incomplete identity between them as shown in Fig 2 and 3.

Molecular docking results:

Molecular docking was done to predict all possible orientations of the tested compounds and to find out a possible explanation of the activity of all compounds against *Bacillus Subtilis*. According to the literature it was reported that acridine derivatives have a high affinity of binding in the DNA gate of bacterial gyrase [41-43].

DNA gate has a catalytic Tyrosine residues (Tyr 123) that acts as nucleophiles by their hydroxyl groups to attack the DNA and participate in the DNA cleavage process. In the catalytic area of DNA gate there are also some other residues that interacted together for the integrity of the DNA binding site such as; Ala 68, Gly 72, Gly 76, Tyr 150 and Arg 69. These residues enable the gate to form a close as well.

The ability of any compound to interact with the Tyr 123 (the main catalytic residue) will inhibit the DNA binding. Also, the blocking of DNA gate by planar structures like acridines may help in that as well.

According to the docking results it was observed that compound 1 interacted by a hydrogen bond that is formed between its C=N and –OH of Tyr 123. Compound 2A had two modes of binding; one by a hydrophobic interactions with Arg 122 and the second by hydrogen bond with Tyr 123. The same was observed with compounds 2B and 2C. Compound 2D participated by its nitrile group to form a hydrogen bond with Tyr 123. Compound 5c had hydrophobic interactions due to the $\pi - \pi$ interactions formed by its p-chlorophenyl ring. Compound 6A showed a hydrogen bond by its pyrazole –NH with Arg 122.

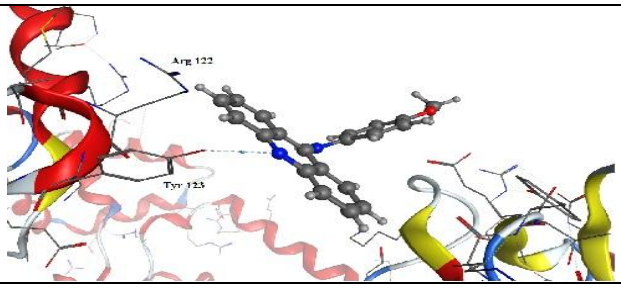
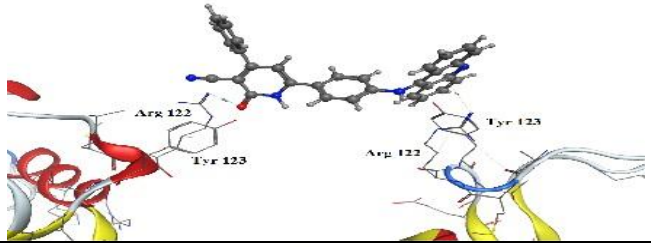
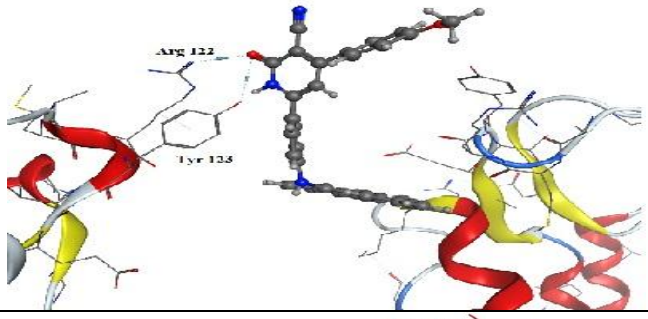
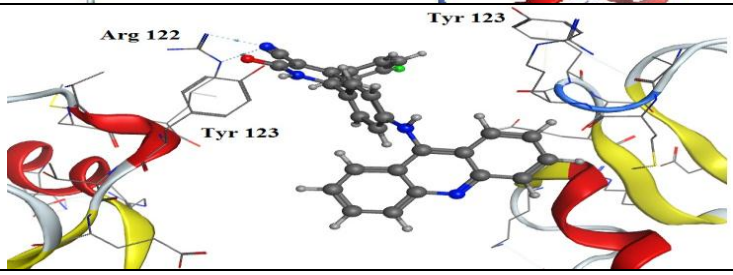
It is also predicted that all compounds have nitrile group may interact by a covalent bond with the Tyr nucleophilic center (Table 2 shows all docking results).

Table 1. The antimicrobial screening of some new acridine compounds

Comp. No.	*B. Aublilis*	*E. Coli*	*S. Aureus*	*S.Lutea*	*P.Aeruginosa*	*M. Plieli*	C. *Albican*	*A. Niger*
1	+++	+++	-	-	-	.		-
2a	+++++		-	-	-	-	-	-
2b	+++++	-	++++	-	-	-	-	-
2c	+++++	-	-	-	-	-	-	-
2d	+++++	-	+	-	-	-	-	-
2e	+++++	-	-	-	-	++++	-	-
5c	++++	-	-	-	-	-	-	-
6a	+++++	+	-	-	-	-	-	++++
NA	+++++	+++++	+++++	+++	+	++	-	(-)
Clotrimazole	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+++++)

+++++ = highly significant ++++ = moderately significant
+++ = slight significant ++ = fairly significant
+ = weakly significant - = inactive (-) = not test.

Table 2. Docking of all tested compounds against Bacillus subtilis gyrase

		Affinity Kcal/mol	Residues
1		-11.84	Arg 122 and Tyr 123
2A		-12.35	Arg 122
2B		-12.25	Arg 122 and Tyr 123
2C		-12.40	Arg 122 and Tyr 123

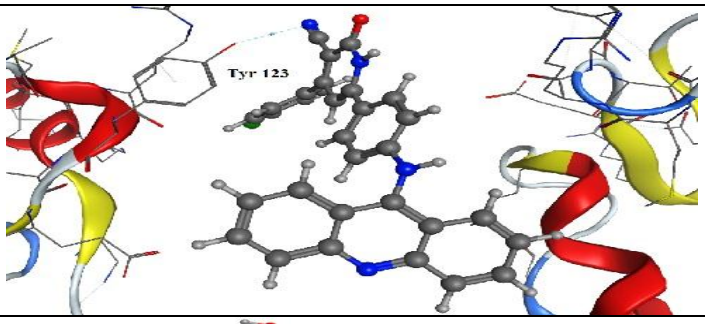
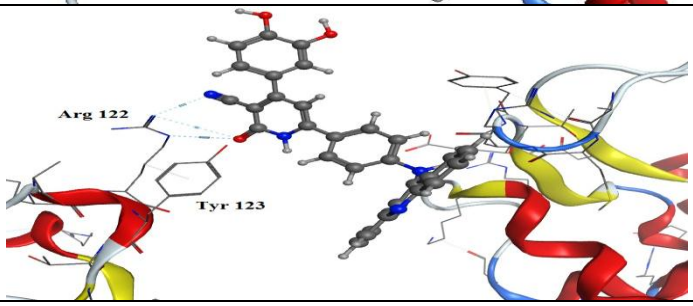
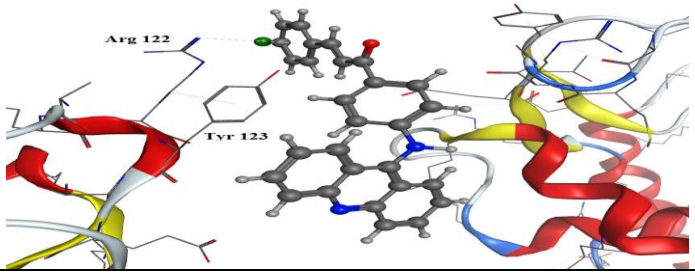
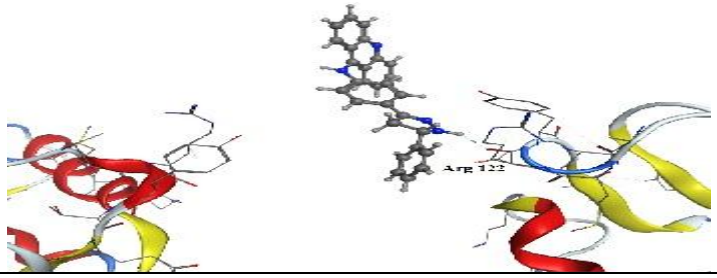
2D		-11.95	Arg 122 and Tyr 123
2E		-12.33	Arg 122 and Tyr 123
5C		-12.12	Arg 122 and Tyr 123
6A		-11.75	Arg 122

Figure 1. Chemical structures: 1, Nitracrine, 2, Acrinal

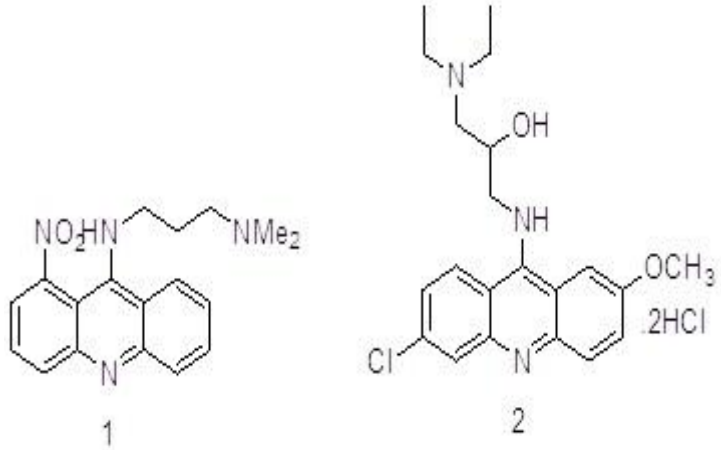
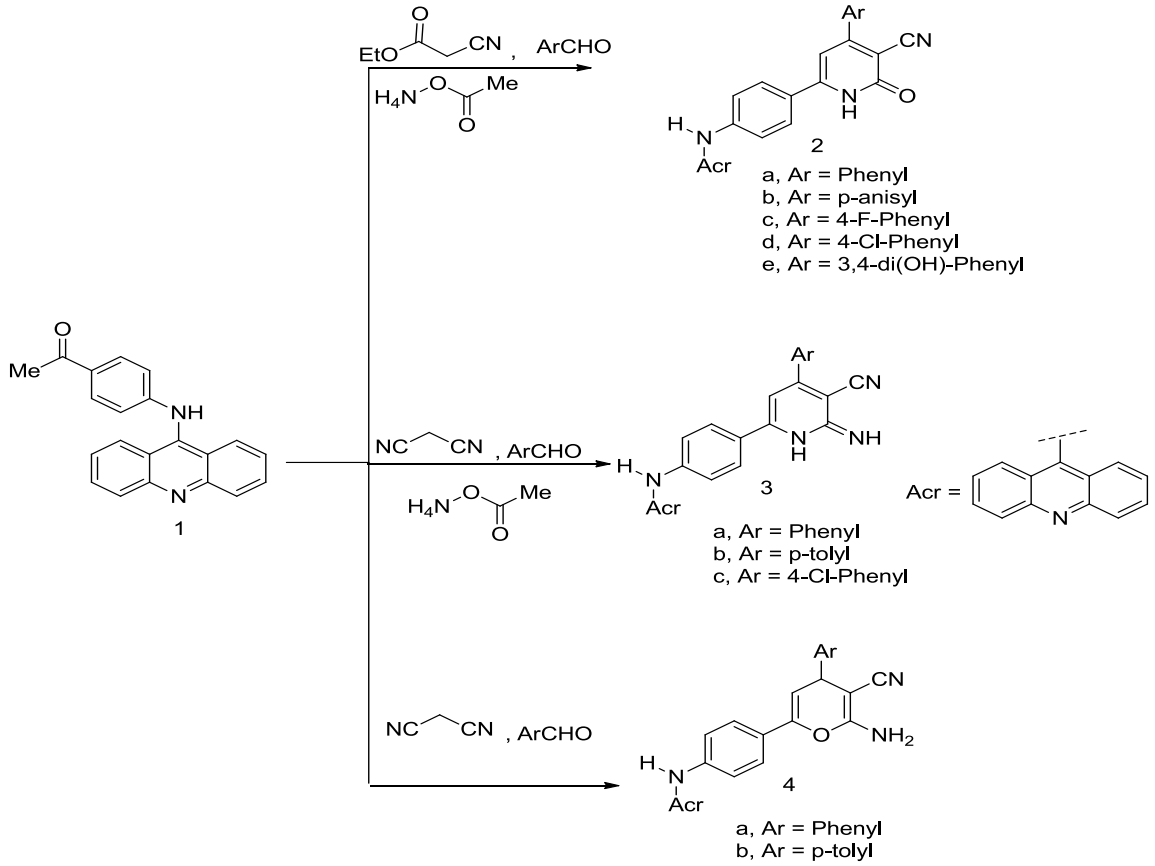


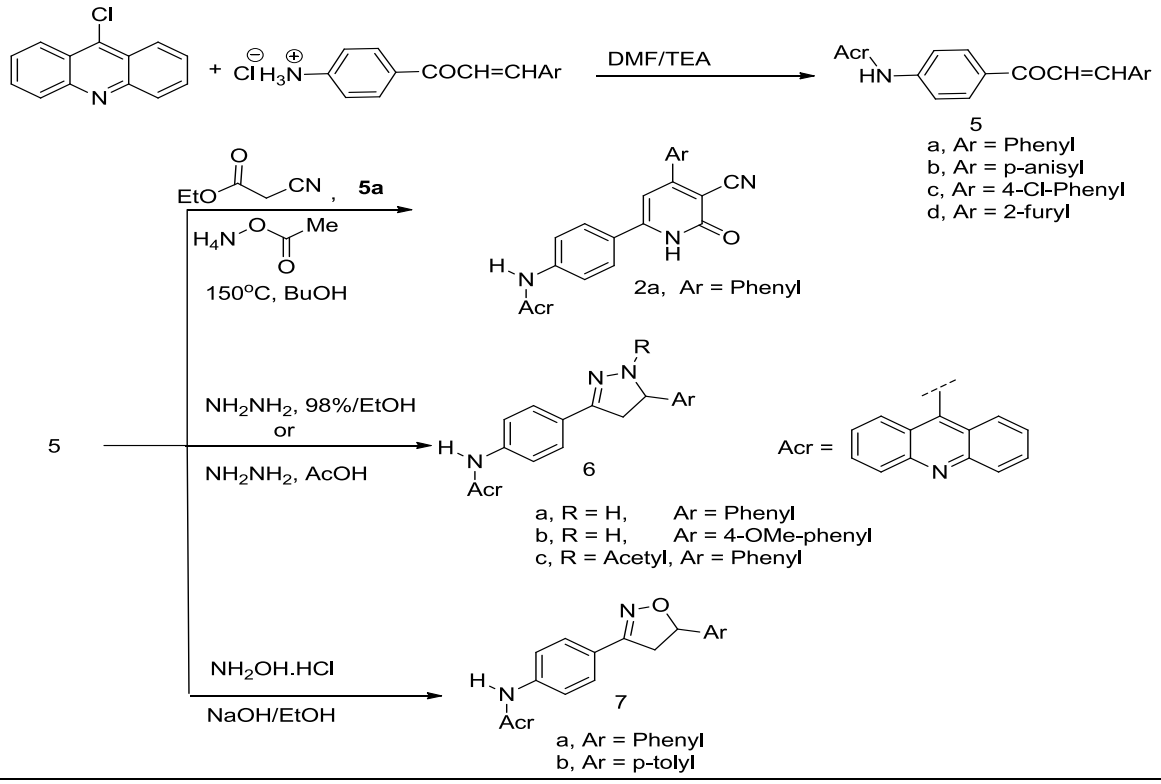
Figure 2. Molecular alignment between the gyrase enzyme of Bacillus subtilis (Uniprot code = P39814) and E-coli (uniprot code = P06612) with identity percentage = 33%

Figure 3. Molecular alignment between the gyrase enzyme of Bacillus subtilis (Uniprot code = P39814) and Staph. aerus (uniprot code = Q2FHI8) with identity percentage = 67.43%.

Scheme 1



Scheme 2



CONCLUSION

Several compounds with the 9- amino or anilino acridine core were prepared and their anti-microbial properties were studied. The antimicrobial potency of some the newly synthesized compounds were subjected for further docking studies to explore the binding pattern against *Bacillus Subtilis* (gram positive bacteria).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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