European Journal of Medicinal Chemistry 56 (2012) 217–224

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Docking studies, synthesis, characterization of some novel oxazine substituted 9-anilinoacridine derivatives and evaluation for their antioxidant and anticancer activities as topoisomerase II inhibitors

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ARTICLE INFO

Article history:
Received 8 June 2012
Received in revised form
15 August 2012
Accepted 17 August 2012
Available online 31 August 2012

Keywords:
Acridine
Chalcone
Oxazine
Antioxidant
Anticancer

ABSTRACT

A series of 9-anilinoacridines substituted with oxazine derivatives were synthesized to evaluate their antioxidant and anticancer activity against Daltons Lymphoma Ascites (DLA) cell growth by *in vitro* method. It was revealed that these conjugates exhibited significant antioxidant and anticancer activity (inhibition of DLA cell proliferation). Among these agents, compounds **5a**, **5h**, **5i**, **5j** were the most cytotoxic with CTC_{50} value of 140–250 μg/mL. The docking studies of the synthesized compounds were performed towards the key Topoisomerase II (1QZR) by using Schrodinger Maestro 9.2 version. The oxazine substituted 9-anilinoacridine derivatives **5a**, **5h**, **5i**, **5j** have significant anticancer activity as topoisomerase II inhibitors.

1. Introduction

Acridine derivatives are one of the most studied chemotherapeutic compounds, widely used as antimicrobial [1], antioxidant [2], anticancer [3–7], antimalarial [8,9], anti-inflammatory [10], analgesic [11], antileishmanial [12], antinociceptive [13], acetyl cholinesterase inhibitors [14] and antiherpes [15] etc. Antitumour cytotoxic agents with DNA-intercalative properties have been studied. Amsacrine is the best-known compound of 9-anilinoacridines series. It was one of the first DNA-intercalating agents to be considered as a topoisomerase II inhibitor. The intercalation process is the strongest type of reversible binding to the double helical DNA in compounds with sufficiently large coplanar aromatic chromophore. Moreover, the cytotoxicity of most of the clinically useful DNA-intercalating agents involves the inhibition of the enzyme DNA-topoisomerase I or II. Several detailed SAR studies of acridine-based DNA-intercalating agents suggest that the mode of binding is important and the chromophore intercalate with the DNA base pairs. The chemical modification of acridines such as the introduction of different substitutions or hetero cyclic rings were allowed expansion of research on the structure activity relationship to afford new insight into molecular interactions at the receptor level [16]. In fact, it is well established that slight structural modification on 9-anilinoacridines may bring various pharmacological effects. Similarly oxazine derivatives also have various biological activities [17–20] like antimicrobial, anticancer etc. *In vitro* cytotoxicity against Daltons Lymphoma Ascites (DLA) cell lines are described. As a part of our ongoing research on searching new potent cytotoxic agents, we have synthesized 9-anilinoacridine analogues bearing the oxazine residue on anilino rings for anticancer evaluation. The results revealed that the newly synthesized derivatives exhibited significant *in vitro* cytotoxicity.

2. Chemistry

Our synthetic pathway (Scheme 1) was based on the preparation of 9-chloroacridine **2**, which was refluxed with p-amino-acetophenone to yield 1-(4-(acridine-9-ylamino) phenyl)ethanone **3** [10]. The various chalcone substituted 9-anilinoacridines **4a–j** [21] were prepared by the reaction of **3** with various aldehydes and

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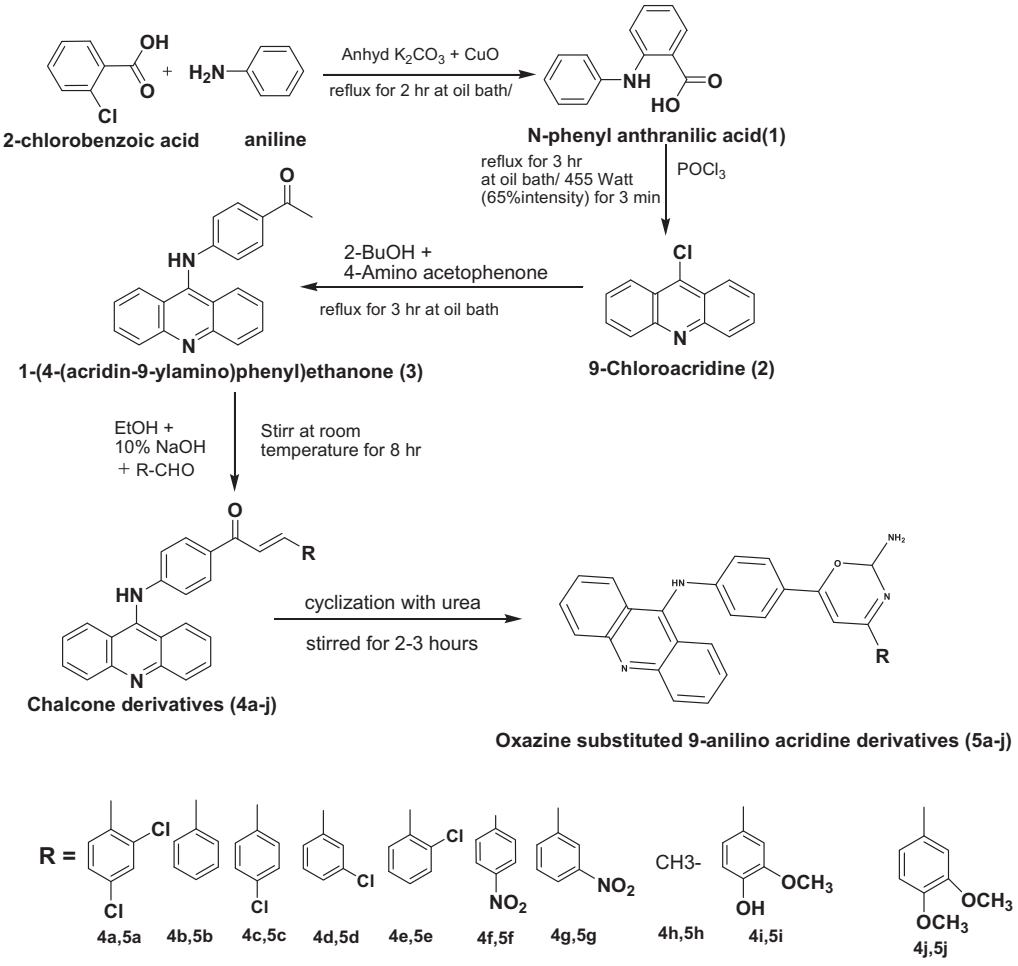
http://dx.doi.org/10.1016/j.ejmech.2012.08.025



Scheme 1.

these chalcone derivatives were allowed to cyclized with urea afford the corresponding oxazine substituted 9-anilinoacridines **5a**–**j** [22]. Synthesis, characterization and evaluation of biological activities of novel chalcone and oxazine substituted 9-anilinoacridines are described in this paper. The synthesized compounds were purified by column chromatography. The final yield of the derivatives was in the range of 50–75%. The compounds obtained were stable in the solid as well as in the solution state. The new compounds were completely characterized by IR, ^{1}H NMR, ^{13}C NMR, mass spectral data and elemental analysis. The IR spectra of compounds **5a**–**j** showed intense bands in the region 1200–1300 cm^{-1} due to carbonyl stretching and broad bands in the region 3200–3400 cm^{-1} due to NH stretching. The ^{1}H NMR spectra also support the structure of the compounds **5a**–**j**. The NH proton appeared at 7.9–8.1 and NH_2 proton at 5.9–6.3. The mass spectra of all compounds **5a**–**j** showed molecular ion peaks confirming their molecular weight.

3. Pharmacology

Acridine derivatives possess a diverse range of pharmacological activities [23–25]. The objectives of this study were to determine whether the oxazine substituted 9-anilinoacridine derivatives posses the antioxidant and cytotoxic activity. Aiming at this goal, we have proved the impact of the acridines on the cytotoxicity of cells by intercalating DNA. Hence all the chalcone and oxazine substituted 9-anilinoacridine derivatives **4a**–**j**, **5a**–**j** were screened for antioxidant activity and **5a**–**j** were screened for short term *in vitro* anticancer activity against Daltons Lymphoma Ascites (DLA) cells. Many of the synthesized final compounds **5a**–**j** have significant activities.

3.1. Antioxidant activity

3.1.1. Scavenging of hydrogen peroxide radicals [26]

Hydrogen peroxide was generated *in vivo* by several oxidase enzymes and by activated phagocytes and it is known to play an important role in the killing of several bacterial and fungal strains. There are increasing evidences that, H_2O_2, either directly or indirectly, OH^{+} can act as a messenger molecule in the synthesis and activation of several inflammatory mediators. When a scavenger is incubated with H_2O_2 using a peroxides assay system, the loss of H_2O_2 can be measured. H_2O_2 itself is a rather weak oxidant and most organic compounds (except for some sulphur containing molecules) were virtually inert to attack by it at ordinary environmental or cellular concentration and temperature.

3.1.2. Scavenging of superoxide radical by alkaline DMSO method [27]

Superoxide was generated *in vitro* and the reduction of NBT by superoxide was determined in the presence and absence of sample and standard shows scavenging ability of different samples and standard against superoxide radicals. The results are shown in Table 1.

Table 1
Antioxidant activity of synthesized compounds **4a–j** and **5a–j** by H_2O_2 method and alkaline DMSO method

S. No.	Compound	IC_{50} values (μg/mL) for H_2O_2 method	IC_{50} values (μg/mL) for alkaline DMSO method
1	**4a**	>1000	>500
2	**4b**	>1000	>500
3	**4c**	>1000	>500
4	**4d**	>1000	>500
5	**4e**	>1000	>500
6	**4f**	>1000	>500
7	**4g**	>1000	>500
8	**4h**	>1000	>500
9	**4i**	>1000	>500
10	**4j**	>1000	>500
11	**5a**	20.03 ± 0.2583	28.02 ± 0.6583
12	**5b**	17.23 ± 1.148	22.27 ± 0.5308
13	**5c**	30.01 ± 0.028	68.13 ± 0.4144
14	**5d**	36.01 ± 0.1028	37.43 ± 0.3296
15	**5e**	25.01 ± 1.123	32.05 ± 0.4825
16	**5f**	24.03 ± 0.1402	47.50 ± 0.5518
17	**5g**	12.00 ± 0.5660	29.03 ± 1.265
18	**5h**	14.20 ± 0.3564	26.30 ± 0.1219
19	**5i**	14.50 ± 0.2764	24.02 ± 0.3567
20	**5j**	18.06 ± 0.6568	28.01 ± 0.504
21	Standard ascorbic acid	18.60 ± 1.039	24.77 ± 0.2404

3.2. Short term study for antitumour activity [28]

Short term antitumour activity of the compounds were assayed by determining the percentage viability of DLA cells using trypan blue dye exclusion technique. DLA cells were cultured in the peritoneal cavity of healthy albino mice weighing 25–30 g by injecting a suspension of DLA cells (1×10^6 cells/mL) intraperitoneally. The cells were aspirated aseptically from the peritoneal cavity of the mice on day 15. The cells were washed with Hank's balanced salt solution (HBSS) and centrifuged for 10–15 min at 1500 rpm in the cooling centrifuge. The pellet was re-suspended with HBSS and the process was repeated three times. Finally the cells were suspended in a known quantity of HBSS and the cell count was adjusted to 2×10^6 cells/mL. 0.1 mL of the diluted cell suspension was distributed in to Eppendorf tubes and exposed 0.1 mL each of the different concentration of the drug in phosphate buffer saline and incubated at 37 °C, 5% CO_2 for 3 h. After 3 h, trypan blue dye exclusion test was performed to determine percentage viability.

For testing viability using dye exclusion method, the pooled cells from wells of each concentration were mixed with 0.4% trypan blue in a ratio of 1:1 and the number of stained, non-stained and total number of cells were counted using haemocytometer. The percentage inhibition and CTC_{50} values were calculated.

4. Docking studies [29]

Protein preparation was performed by using protein preparation Wizard of Maestro 9.2 software. The protein structure 1*QZR* was taken from PDB data bank. The A chain was treated to add missing hydrogen, assign proper bond orders and delete water molecules which were more than 5 Å from the heterogeneous groups. Finally, the protein structure was minimized to the default Root Mean Square Deviation (RMSD) value of 0.30.

Ligand preparation was accomplished on all the 10 compounds using Ligprep module to clean the structure and generate tautomers as described.

Receptor was defined and the co-crystallized ligand was differentiated from the active site of receptor A chain. The atoms were scaled by Van der Waals radii of 1.0 Å with the partial atomic charge less than 0.25 defaults. The active site was defined as an enclosing box at the centroid of the workspace ligand as selected in the receptor folder. The ligands similar in size to the workspace ligand were allowed to dock into the active site. No constraints either positional, H-bonding or hydrophobic were defined.

Ligand docking was performed using OPLS force field. The receptor grid defined in the receptor grid generation folder was selected for the docking of ligands prepared by using Ligprep. Flexible docking was performed using the Extra Precision (XP) feature of Glide module. The structure output format was set to pose viewer file so as to view the output of the resulting docking studies from pose-viewer. The XP-Glide predicted pose of active compounds in the scheme are shown in Fig. 1.

5. Results and discussion

Results are summarized in Tables 1–3 and schematised in Scheme 1. They showed that the pharmacological properties of the compounds greatly depended on the number and the chemical nature of the substituents.

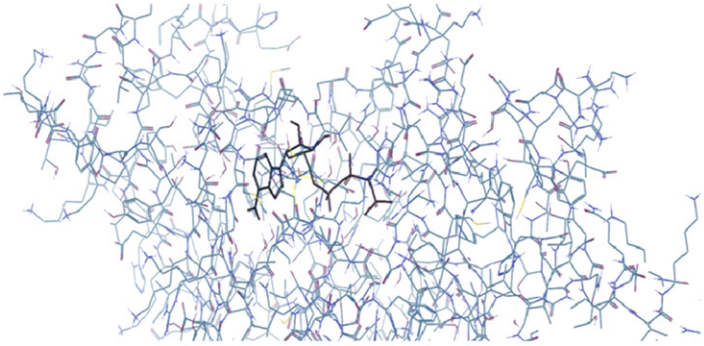
For *in vitro* antioxidant activity, all the synthesized chalcone and oxazine substituted 9-anilinoacridines were screened by H_2O_2 method and alkaline DMSO method. Almost all the oxazine substituted derivatives exerted an interesting antioxidant activity. Among these compounds **5a**, **5b**, **5g**, **5h**, **5i** and **5j** were more potent antioxidant activity when compared to standard ascorbic acid. The IC_{50} values, which are the concentration of the sample required to increase 50% of reducing capacity of the compounds are summarized in Table 1.

The docking studies of the ligands to protein active sites were performed by an advanced molecular docking program Schrodinger Maestro 9.2 version for determining the binding affinities of the compounds. The designed analogues are docked towards the Topoisomerase II (1QZR) in order to ascertain their anticancer activity. The analogues show best fit Root Mean Square Difference (rmsd) value of 0.000. The results obtained in the molecular docking studies showed a good correlation between their short term *in vitro* anticancer activity and Glide score. The compounds **5h**, **5i**, **5j** showed good affinity to the receptor due to more lipophilic character and hydrogen bonding when compared with standard. The results are summarized in the Table 2.

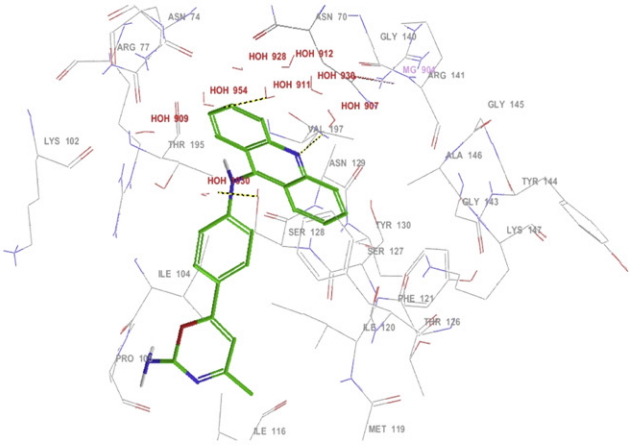
The synthesized final compounds **5a–j** were subjected to short term study for *in vitro* antitumour activity against Daltons Lymphoma Ascites (DLA) cells. The compounds **5a**, **5h**, **5i**, **5j** exerted significant anticancer activity against DLA cells at the concentration of 140–250 μg/mL (0.352–0.512 μM). The results are summarized in the Table 3. The aliphatic methyl at 4th position of oxazine increases the cytotoxic activity than aromatic substitution. 4th position of oxazine has di substituted aromatic ring which increases the cytotoxic activity than mono substituted aromatic ring.

The compounds **5h**, **5i** and **5j** exhibited the highest Glide score −5.37, −6 and −6.22 respectively are having good *in vitro* anticancer activity. The best affinity modes of the docked compounds (**5a**, **5h**, **5i**, **5j**) with Topoisomerase II were shown in Fig. 1.

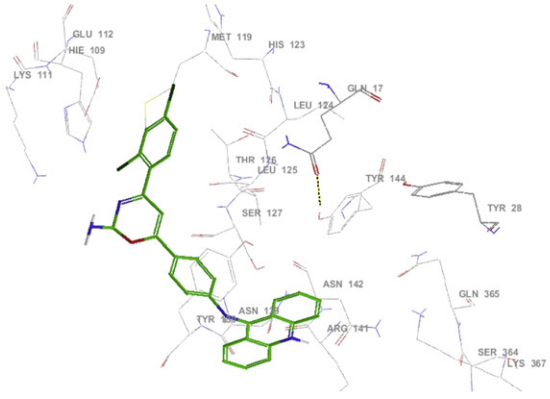
The compound **5j** has more hydrogen bonding with receptor and lipophilic factors for their good affinity to the receptor site which was shown in the Fig. 2. The compounds **5h** and **5i** also have more lipophilic factors for their good affinity to the receptor site. So this research work can be used for further study to synthesize many useful medicinal compounds.



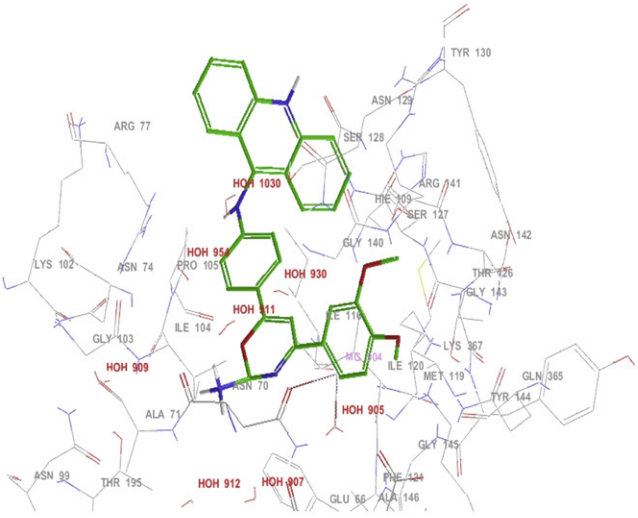
Topo isomerase II (1QZR) chain A with ligand



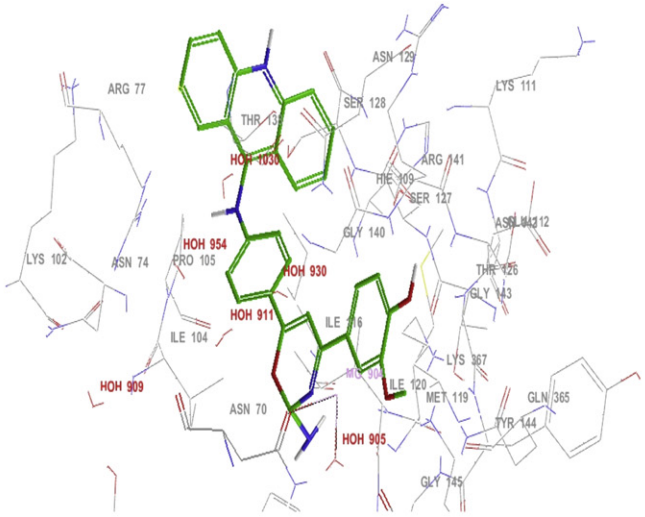
Compound 5i with topo isomerase II (1QZR)



Compound 5a with topo isomerase II (1QZR)



Compound 5j with topo isomerase II (1QZR)



Compound 5h with topo isomerase II (1QZR)

Fig. 1. Docking pictures of compounds with topoisomerase II (1QZR).

6. Conclusion

Acridine family includes a wide range of tricyclic molecules with various biological properties. Considered as potential anticancer agents since the 1980s, numerous acridine derivatives have been synthesised and successfully assessed for their anticancer activity. On this basis, authors recently demonstrated that diverse compounds of the oxazine substituted 9-anilinoacridine series exerted potent anticancer activities. It was revealed that these agents exhibited significant cytotoxicity against DLA cell growth *in vitro* as Topoisomerase II inhibitor. Results observed in the present study clearly demonstrated that some derivatives of the oxazine substituted 9-anilinoacridine family could exert interesting anticancer and antioxidant activities. The molecular docking studies show a good correlation between their biological activities screened and Glide score. The compounds **5a**, **5h**, **5i**, **5j** have

Table 2
Docking studies of synthesized compounds with Topoisomerase II (1QZR).

S. No.	Compound no	G-score	Lipophilic Evdw	H-bond
1	**5a**	−3.45	−2.13	−0.03
2	**5b**	−5.26	−3.48	−0.69
3	**5c**	−5.44	−3.86	−0.42
4	**5d**	−5.41	−2.2	−1.26
5	**5e**	−5.05	−3.45	−0.83
6	**5f**	−4.50	−3.39	0
7	**5g**	−3.3	−3.68	0
8	**5h**	**−5.37**	−4.68	−0.52
9	**5i**	**−6**	−4.53	−0.91
10	**5j**	**−6.22**	−3.91	−1.35
11	Ledacrine (std)	−5.13	−3.95	−0.53

significant anticancer activity and are likely to be useful as drugs after further refinement. These derivatives will encourage helping to design future anticancer agents with therapeutic potentials.

7. Experimental protocol

Melting points were obtained on Veego VMP-1 apparatus in open capillary tubes and are uncorrected. The reactions were monitored by TLC on silica gel thin layer plates. Compounds were analysed for C, H, N and analytical results obtained for these elements were within ±0.5% of the calculated values for the formula shown. All reagents were of commercial quality or were purified before use. Organic solvents were of analytical grade or were purified by standard procedures. IR spectra were obtained using a Shimadzu 8400 FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR were recorded on Bruker A VIII 500 MHz Spectrometer. Chemical shifts are in parts per million (ppm). Mass spectra of the final compounds were recorded on a JEOL GC mate Mass Spectrometer.

7.1. Synthesis and characterization of the compounds

7.1.1. Synthesis of 9-chloroacridine **2**

9-Chloroacridine was synthesized by the cyclization of *N*-arylanthranilic acid with phosphorus oxychloride as reported [21].

7.1.2. Synthesis of 1-[4-(acridin-9-ylamino)phenyl]ethanone **3**

In a 250 mL round bottomed flask 4.06 g (0.03 mol) of 4-aminoacetophenone was refluxed with 5.4528 g (0.0256 mol) of 9-chloroacridine in 80 mL of 2-butanol for 3 h [10]. After completion of reaction the reaction mixture was allowed to cool to room temperature then it was poured into 150 mL of ice water. A precipitate formed was filtered by suction, washed with water, dried and recrystallized from ethanol.

Table 3
Short term *in vitro* anticancer activity of synthesized compounds against Daltons Lymphoma Ascites (DLA) cells.

S. No.	Compound name	CTC_{50} (μg/mL)
1	**5a**	**180**
2	**5b**	425
3	**5c**	640
4	**5d**	820
5	**5e**	590
6	**5f**	725
7	**5g**	600
8	**5h**	**140**
9	**5i**	**250**
10	**5j**	**190**

CTC_{50} – concentration required to reduce viability by 50%.

7.2. General procedure for synthesis of chalcones ***4a–j***

The chalcones were synthesized by using general Claisen–Schimdt condensation [21]. In a 100 mL flat bottomed flask 25 mL of the 10% sodium hydroxide and 25 mL of ethanol were taken with a magnetic stirring bar and it was stirred on the magnetic stirrer. To this 0.01 mol of corresponding aldehyde was added, then 2.99 g (0.0096 mol) of 1-[4-(acridin-9-ylamino)phenyl] ethanone was added at the last. The solution was allowed to stir for 8 h at room temperature. After completion of the reaction 100 mL of water was added, formed precipitate was filtered washed three times with 50 mL of water each time to remove sodium hydroxide, dried and crystallized from ethanol.

7.2.1. (E)-1-(4-(Acridin-9-ylamino)phenyl)-3-(2,4-dichloro phenyl) prop-2-en-1-one (***4a***)

This compound was obtained as a yellow powder; m.p.: 195–197 °C; Yield: 79%; Anal. Calc. for $C_{28}H_{18}Cl_2N_2O$: C, 71.68; H, 3.73; N, 5.90; Found: C, 71.73; H, 3.77; N, 5.92; IR (KBr, υ, cm^{-1}): 3269 (N–H), 3063–3000 (Ar C–H), 1649 (α,β-unsat. C=O), 1607 & 1512 (Ar C=C), 1231 (C–N), 761 (Ar C–H); MS: *m*/*z* 468.16 (M^+); ^{1}H NMR (in ppm): 6.65–8.02 (m, ArH), 7.90 and 7.56 (s, α,β-unsaturated), 11.21 (s, NH); ^{13}C NMR (in ppm) :184.4, 153.5, 150.8, 143.2, 141.2, 136.3, 136.1, 132.4, 131.7, 130.8, 129.6, 130.5, 128.6, 127.2, 127.1, 127.2, 121.6, 119.5, 116.3.

7.2.2. (E)-1-(4-(Acridin-9-ylamino)phenyl)-3-phenyl prop-2-en-1-one (***4b***)

This compound was obtained as a yellow powder; m.p.: 179–181 °C; Yield: 78%; Anal. Calc. for $C_{28}H_{20}N_2O$: C, 83.98; H, 5.03; N, 7.07; Found: C, 83.84; H, 5.12; N, 7.13; IR (KBr, υ, cm^{-1}): 3269 (N–H), 3057–3000 (Ar C–H), 1647 (α,β-unsat. C=O), 1604 & 1516 (Ar C=C), 1226 (C–N), 759 (Ar C–H); MS: *m*/*z* 400.16 (M^+); ^{1}H NMR (in ppm): 6.65–8.02 (16H, m, ArH), 7.90 and 7.56 (2H, s, α,β-unsaturated), 11.21 (1H, s, NH); ^{13}C NMR (in ppm) :183.4, 153.8, 149.8, 142.8, 140.5, 136.1, 135.4, 131.7, 131.3, 130.4, 129.3, 129.8, 128.9, 127.8, 126.7, 126.7, 121.9, 119.3, 115.7.

7.2.3. (E)-1-(4-(Acridin-9-ylamino)phenyl)-3-(4-chlorophenyl) prop-2-en-1-one (***4c***)

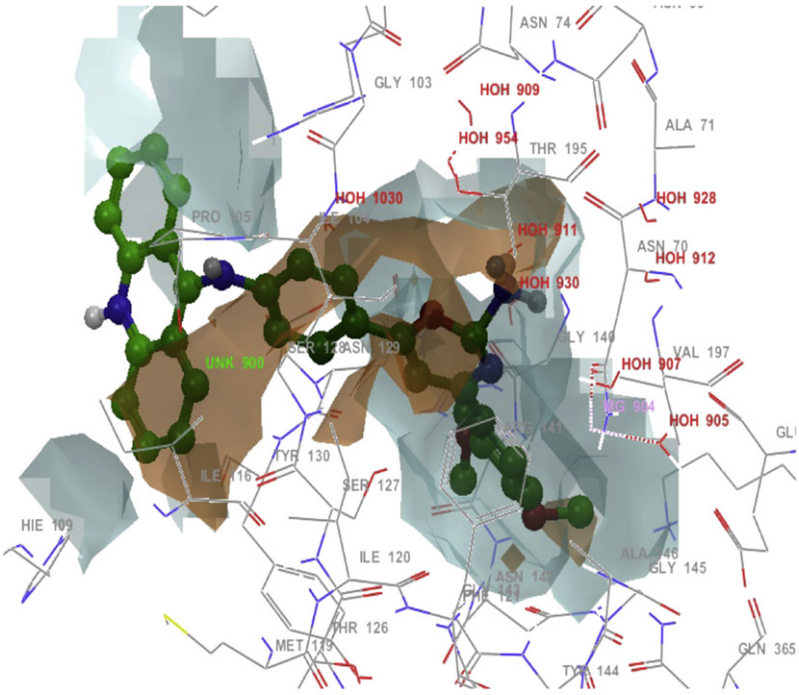
This compound was obtained as a yellow powder; m.p.: 188–190 °C; Yield: 65%; Anal. Calc. for $C_{28}H_{19}ClN_2O$: C, 77.33; H, 4.40; N, 6.44; Found: C, 77.25; H, 4.29; N, 6.62; IR (KBr, υ, cm^{-1}): 3302 (N–H), 3100–3000 (Ar C–H), 1651 (α,β-unsaturated C=O), 1606 & 1518 (Ar C=C), 1267 (C–N), 748 (Ar C–H); MS: *m*/*z* 434.12 (M^+); ^{1}H NMR (in ppm): 6.65–8.02 (16H, m, ArH), 7.90 and 7.56 (2H, s, α,β-unsaturated carbonyl), 11.21 (1H, s, NH); ^{13}C NMR (in ppm) :187 (C=O), 114.8–149.7 (aromatic carbons).

7.2.4. (E)-1-(4-(Acridin-9-ylamino)phenyl)-3-(3-chlorophenyl) prop-2-en-1-one (***4d***)

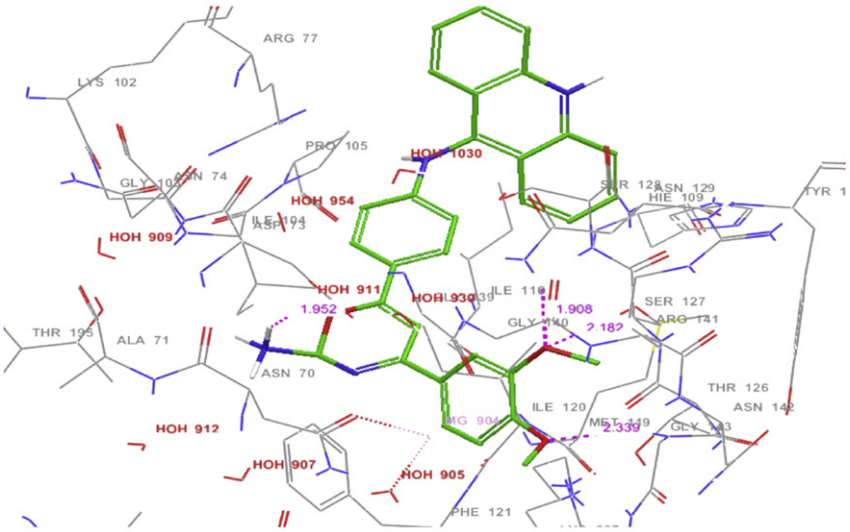
This compound was obtained as a yellow powder; m.p.: 196–198 °C; Yield: 65%; Anal. Calc. for $C_{28}H_{19}ClN_2O$: C, 77.32; H, 4.42; N, 6.45; Found: C, 77.25; H, 4.29; N, 6.62; IR (KBr, υ, cm^{-1}): 3302 (N–H), 3100–3000 (Ar C–H), 1624 (α,β-unsat. C=O), 1606 & 1518 (Ar C=C), 1267 (C–N), 748 (Ar C–H); MS: *m*/*z* 434.52 (M^+); ^{1}H NMR (in ppm): 6.65–8.02 (16H, m, ArH), 7.90 and 7.56 (2H, s, α,β-unsaturated carbonyl), 11.21 (1H, s, NH); ^{13}C NMR (in ppm) :189 (C=O), 114.8–149.7 (aromatic carbons).

7.2.5. (E)-1-(4-(Acridin-9-ylamino)phenyl)-3-(2-chlorophenyl) prop-2-en-1-one (***4e***)

This compound was obtained as a cherry red powder; m.p.: 190–192 °C; Yield: 53%; Anal. Calc. for $C_{28}H_{19}ClN_2O$: C, 77.33; H, 4.40; N, 6.43; Found: C, 77.59; H, 4.20; N, 6.25; IR (KBr, υ, cm^{-1}): 3279 (N–H),



Liphophilic evidence for compound 5j



H- bonding for compound 5j

Fig. 2. Lipophilic evidence and hydrogen bonding for compound **5j**.

3109–2999 (Ar C–H), 1647 (α,β-unsat. C=O), 1591 & 1496 (Ar C=C), 1273 (C–N), 746 (Ar C–H); MS: *m*/*z* 434.62 (M^+); ^{1}H NMR (in ppm): 6.65–8.02 (16H, m, ArH), 7.90 and 7.56 (2H, s, α,β-unsaturated carbonyl), 11.21 (1H, s, NH); ^{13}C NMR (in ppm) :189 (C=O), 114.8–149.7 (aromatic carbons).

*7.2.6. (E)-1-(4-(Acridin-9-ylamino)phenyl)-3-(4-nitro phenyl) prop-2-en-1-one (**4f**)*

This compound was obtained as a yellow powder, m.p.: 208–210 °C; Yield: 65%; Anal. Calc. for $C_{28}H_{19}N_3O_3$: C, 75.49; H, 4.32; N, 9.45; Found: C, 75.26; H, 4.52; N, 9.51; IR (KBr, υ, cm^{-1}): 3034 (N–H), 3100–3000 (Ar C–H), 1622 (α,β-unsat. C=O), 1577 & 1498 (Ar C=C), 1529 & 1348 (NO_2), 1280 (C–N), 748 (Ar C–H); MS: *m*/*z* 445.34 (M^+); ^{1}H NMR (in ppm): 6.65–8.02 (16H, m, ArH), 7.90 and 7.56 (2H, s, α,β-unsaturated carbonyl), 11.21 (1H, s, NH); ^{13}C NMR (in ppm) :189 (C=O), 114.8–149.7 (aromatic carbons).

*7.2.7. (E)-1-(4-(Acridin-9-ylamino)phenyl)-3-(3-nitro phenyl) prop-2-en-1-one (**4g**)*

This compound was obtained as a yellow powder; m.p.: 188–190 °C; Yield: 65%; Anal. Calc. for $C_{28}H_{19}N_3O_3$: C, 75.47; H, 4.30; N, 9.45; Found: C, 75.26; H, 4.52; N, 9.51; IR (KBr, υ, cm^{-1}): 3034 (N–H), 3100–3000 (Ar C–H), 1626 (α,β-unsat. C=O), 1577 & 1498 (Ar C=C), 1529 & 1348 (NO_2), 1280 (C–N), 748 (Ar C–H); MS: *m*/*z*

445.37 (M^+); ^{1}H NMR (in ppm): 6.65–8.02 (16H, m, ArH), 7.90 and 7.56 (2H, s, α,β-unsaturated carbonyl), 11.21 (1H, s, NH); ^{13}C NMR (in ppm) :189 (C=O), 114.8–149.7 (aromatic carbons).

*7.2.8. (E)-1-(4-(Acridin-9-ylamino)phenyl)-3-(4-hydroxy-3-methoxy phenyl)prop-2-en-1-one (**4h**)*

This compound was obtained as a yellow powder; m.p.: 210–212 °C; Yield: 73%; Anal. Calc. for $C_{29}H_{26}N_2O_3$: C, 77.42; H, 5.85; N, 6.33; Found: C, 77.46; H, 5.92; N, 6.37; IR (KBr, υ, cm^{-1}): 3304 (N–H), 3100–3000 (Ar st C–H), 1626 (α,β-unsat. C=O), 1587 & 1568 (Ar C=C), 3327 (Ar–OH), 748 (Ar C–H); MS: *m*/*z* 450.34 (M^+); ^{1}H NMR (in ppm): 6.65–8.02 (16H, m, ArH), 7.90 and 7.56 (2H, s, α,β-unsaturated carbonyl), 11.21 (1H, s, NH); ^{13}C NMR (in ppm) :176 (C=O), 42.03 (OCH_3), 110.2–169.3 (aromatic carbons).

*7.2.9. (E)-1-(4-(Acridin-9-ylamino)phenyl)but-2-en-1-one (**4i**)*

This compound was obtained as an orange powder; m.p.: 188–190 °C; Yield: 61%; Anal. Calc. for $C_{23}H_{18}N_2O$: C, 81.49; H, 5.33; N, 8.23; Found: C, 81.53; H, 5.37; N, 8.13; IR (KBr, υ, cm^{-1}): 3347 (N–H), 3100–3000 (Ar st C–H), 1622 (α,β-unsat. C=O), 1604 & 1473 (Ar C=C); MS: *m*/*z* 338.18 (M^+); ^{1}H NMR (in ppm): 6.65–8.02 (16H, m, ArH), 7.90 and 7.56 (2H, s, α,β-unsaturated carbonyl), 11.21 (1H, s, NH); ^{13}C NMR (in ppm) :189 (C=O), 3.72 (CH_3), 114.8–149.7 (aromatic carbons).

*7.2.10. (E)-1-(4-(Acridin-9-ylamino)phenyl)-3-(3,4-dimethoxy phenyl)prop-2-en-1-one (**4j**)*

This compound was obtained as a yellow powder; m.p.: 228–230 °C; Yield: 54%; Anal. Calc. for $C_{30}H_{24}N_2O_3$: C, 78.29; H, 5.33; N, 6.13; Found: C, 78.25; H, 5.36; N, 6.12; IR (KBr, υ, cm^{-1}): 3044 (N–H), 3100–3000 (Ar st C–H), 1626 (α,β-unsaturated C=O), 1577 & 1498 (Ar C=C), 748 (Ar C–H); MS: *m*/*z* 460.58 (M^+); ^{1}H NMR (in ppm): 6.65–8.02 (16H, m, ArH), 7.90 and 7.56 (2H, s, α,β-unsaturated carbonyl), 11.21 (1H, s, NH). ^{13}C NMR (in ppm):181 (C=O), 53.49 (OCH_3), 54.31 (OCH_3), 104.8–158.6 (aromatic carbons).

*7.3. General procedure for synthesis of oxazine substituted 9-anilinoacridines **5a**–**j***

A mixture of chalcone **4a**–**j** (0.02 mol), urea (0.02) were dissolved in ethanolic sodium hydroxide (10 mL) was stirred about for 2–3 h with a magnetic stirrer. This was then poured into 400 mL of cold water with continuous stirring for an hour. This was kept in refrigerator for 24 h. The precipitate obtained was filtered, washed and recrystallized using petroleum ether:benzene (5:5). The reaction was monitored by TLC using methanol:water (5:3).

*7.3.1. N-(4-(2-Amino-4-(2,4-dichloro phenyl)-2H-1,3-oxazin-6yl) phenyl)acridin-9-amine (**5a**)*

This compound is obtained as a yellow powder; m.p.: 170–172 °C; Yield: 56%; Anal. calc. for $C_{29}H_{20}Cl_2N_4O$: C, 68.24; H, 3.87; N, 13.86; Found: C, 68.18; H, 3.82; N, 13.78; IR (KBr, υ, cm^{-1}): 3340 (N–H), 2993 (Ar st C–H), 1600 & 1473 (Ar C=C), 1157 (Ar C=N), 1226 (C–O), 742 (Ar C–H); MS: *m*/*z* 511.15 (M^+); ^{1}H NMR (in ppm): 6.85–7.94 (16H, m, ArH), 7.95 (1H, s, NH), 6. 19 (2H, s, NH_2); ^{13}C NMR (in ppm): 114.3–178.4 (aromatic carbons).

*7.3.2. N-(4-(2-Amino-4-(phenyl)-2H-1,3-oxazin-6yl)phenyl) acridin-9-amine (**5b**)*

This compound is obtained as a Brownish yellow powder; m.p.: 215–217 °C; Yield: 78%; Anal. calc. for $C_{29}H_{22}N_4O$: C, 78.74; H, 5.17; N, 12.57; Found: C, 78.78; H, 5.23; N, 12.67; IR (KBr, υ, cm^{-1}): 3340 (N–H), 2991 (Ar st C–H), 1600 & 1473 (Ar C=C), 1157 (Ar C=N), 1226 (CO), 742 (Ar C–H); MS: *m*/*z* 442.57 (M^+); ^{1}H NMR (in ppm): 6.83–8.92 (16H, m, ArH), 7.95 (1H, s, NH), 6. 19 (2H, s, NH_2); ^{13}C NMR (in ppm): 112.3–166.1 (aromatic carbons).

*7.3.3. N-(4-(2-Amino-4-(4-chloro phenyl)-2H-1,3-oxazin-6yl) phenyl)acridin-9-amine (**5c**)*

This compound is obtained as a yellow powder; m.p.: 175–177 °C; Yield: 67%; Anal. calc. for $C_{29}H_{21}ClN_4O$: C, 73.18; H, 4.45; Cl, 7.52; N, 11.68; Found: C, 73.12; H, 4.49; Cl, 7.56; N, 11.73; IR (KBr, υ, cm^{-1}): 3218 (N–H), 3008 (Ar st C–H), 1573 & 1545 (Ar C=C), 1157 (Ar C=N), 1227 (C–O), 756 (Ar C–H), 815 (C–Cl); MS: *m*/*z* 476.56 (M^+); ^{1}H NMR (in ppm): 7.36–7.70 (16H, m, ArH), 7.77–8.05 (2H, m, CH), 7.91 (1H, s, NH), 6. 19 (2H, s, NH_2); ^{13}C NMR (in ppm): 113.4–175.1 (aromatic carbons).

*7.3.4. N-(4-(2-Amino-4-(3-chloro phenyl)-2H-1,3-oxazin-6yl) phenyl)acridin-9-amine (**5d**)*

This compound is obtained as a yellowish green powder; m.p.: 185–186 °C; Yield: 75%; Anal. calc. for $C_{29}H_{21}ClN_4O$: C, 73.18; H, 4.45; N, 11.78; Found: C, 73.23; H, 4.48; N, 11.68; IR (KBr, υ, cm^{-1}): 3218 (N–H), 3055 (Ar st C–H), 1606 & 1593 (Ar C=C), 1707 (Ar C=N), 1274 (C–O), 744 (Ar C–H), 831 (C–Cl); MS: *m*/*z* 476.78 (M^+); ^{1}H NMR (in ppm): 6.85–7.97 (18H, m, ArH), 8.02 (1H, s, NH), 6. 21 (2H, s, NH_2); ^{13}C NMR (in ppm): 108.3–157.8 (aromatic carbons).

*7.3.5. N-(4-(2-Amino-4-(2-chloro phenyl)-2H-1,3-oxazin-6yl) phenyl)acridin-9-amine (**5e**)*

This compound is obtained as a green power; m.p.: 132–133 °C; Yield: 66%; Anal. calc. for $C_{29}H_{21}ClN_4O$: C, 73.18; H, 4.38; N, 11.68; Found: C, 73.23; H, 4.28; N, 11.64; IR (KBr, υ, cm^{-1}): 3351 (N–H), 3064 (Ar st C–H), 1593 (Ar C=C), 1707 (Ar C=N), 1274 (C–O), 744 (Ar C–H), 831 (C–Cl); MS: *m*/*z* 476.92 (M^+); ^{1}H NMR (in ppm): 6.24–7.98 (18H, m, ArH), 8.22 (1H, s, NH), 6. 24 (2H, s, NH_2); ^{13}C NMR (in ppm): 108.8–171.4 (aromatic carbons).

*7.3.6. N-(4-(2-Amino-4-(4-nitro phenyl)-2H-1,3-oxazin-6yl) phenyl)acridin-9-amine (**5f**)*

This compound is obtained as a yellow powder; m.p.: 208–209 °C; Yield: 70%; Anal. calc. for $C_{29}H_{21}N_5O_3$: C, 71.37; H, 4.28; N, 14.29; Found: C, 71.41; H, 4.23; N, 14.35; IR (KBr, υ, cm^{-1}): 3463 (N–H), 3080 (Ar st C–H), 1599 & 1583 (Ar C=C), 1635 (Ar C=N), 1232 (C–O), 746 (Ar C–H). MS: *m*/*z* 487.47 (M^+); ^{1}H NMR (in ppm): 6.61–7.21 (18H, m, ArH), 7.94 (1H, s, NH), 6. 02 (2H, s, NH_2); ^{13}C NMR (in ppm): 109.7–168.7 (aromatic carbons).

*7.3.7. N-(4-(2-Amino-4-(3-nitro phenyl)-2H-1,3-oxazin-6yl) phenyl)acridin-9-amine (**5g**)*

This compound is obtained as a yellow powder; m.p.: 210–211 °C; Yield: 61%; Anal. calc. for $C_{29}H_{21}N_5O_3$: C, 71.31; H, 4.29; N, 14.48; Found: C, 71.25; H, 4.42; N, 14.51; IR (KBr, υ, cm^{-1}): 3336 (N–H), 3064 (Ar st C–H), 1589 & 1564 (Ar C=C), 1157 (Ar C=N), 1232 (C–O), 746 (Ar C–H); MS: *m*/*z* 487.47 (M^+); ^{1}H NMR (in ppm): 7.67–7.9 (18H, m, ArH), 8.28 (1H, s, NH), 6. 27 (2H, s, NH_2); ^{13}C NMR (in ppm): 107.8–176.5 (aromatic carbons).

*7.3.8. 4-(6-(4-(Acridin-9-ylamino)phenyl)-2-amino-2H-1,3-oxazin-4yl)-2methoxyphenol (**5h**)*

This compound is obtained as a yellow powder; m.p.: 168–170 °C; Yield: 57%; Anal. calc. for $C_{30}H_{24}N_4O_3$: C, 73.68; H, 4.87; N, 11.36; Found: C, 73.68; H, 4.87; N, 11.36; IR (KBr, υ, cm^{-1}): 3332 (N–H), 2993 (Ar st C–H), 1589 & 1563 (Ar C=C), 3227 (Ar–OH), 1653 (Ar C=N), 1178 (C–O), 750 (Ar C–H); MS: *m*/*z* 488.47 (M^+); ^{1}H NMR (in ppm): 7.27–7.71 (18H, m, ArH), 8.28 (1H, s, NH), 6. 27 (2H, s, NH_2); ^{13}C NMR (in ppm): 40.03 (OCH_3), 112.4–163.9 (aromatic carbons).

*7.3.9. N-(4-(2-Amino-4-(methyl)-2H-1,3-oxazin-6yl)phenyl) acridin-9-amine (**5i**)*

This compound is obtained as a yellow powder; m.p.: 175–177 °C; Yield: 51%; Anal. calc. for $C_{24}H_{20}N_4O$: C, 75.75; H, 5.34; N, 14.68; Found: C, 75.68; H, 5.41; N, 14.62; IR (KBr, υ, cm^{-1}): 3343 (N–H), 3067 (Ar st C–H), 1589 & 1563 (Ar C=C), 1159 (Ar C=N), 1178 (C–O), 742 (Ar C–H); MS: *m*/*z* 380.47 (M^+); ^1H NMR (in ppm): 7.23–7.85 (18H, m, ArH), 8.38 (1H, s, NH), 6. 18 (2H, s, NH_2); ^{13}C NMR (in ppm): 2.50 (CH_3), 112.8–175.2 (aromatic carbons).

*7.3.10. N-(4-(2-Amino-4-(3,4-dimethoxyphenyl)-2H-1,3-oxazin-6yl)phenyl)acridin-9-amine (**5j**)*

This compound is obtained as a dark yellow powder; m.p.: 160–161 °C; Yield: 71%; Anal. calc. for $C_{31}H_{26}N_4O_3$: C, 74.18; H, 5.16; N, 11.12; Found: C, 74.23; H, 5.12; N, 11.18; IR (KBr, υ, cm^{-1}): 3352 (N–H), 3005 (Ar st C–H), 1600 & 1583 (Ar C=C), 1642 (Ar C=N), 1263 (C–O), 744 (Ar C–H); MS: *m*/*z* 502.52 (M^+); ^1H NMR (in ppm): 6.85–7.97 (16H, s, ArH), 7.48 (1H, s, NH), 6. 12 (2H, s, NH_2), 3.80, 3.86 (6H, d, OCH_3); ^{13}C NMR (in ppm): 55.69 (OCH_3), 55.53 (OCH_3), Other aromatic carbons are 110.49, 111.54, 112.7, 119.97, 123.21, 125.55, 127.97, 130.98, 141.87, 148.97, 150.71, 153.66, 185.96.

Acknowledgement

We thank All India Council for Technical Education, New Delhi for the financial support under Research Promotion Scheme. We also thank our Vice Chancellor Dr.B. Suresh, JSS University, Mysore, Department of Pharmaceutical analysis, Department of Pharmaceutical Biotechnology, Department of Pharmacology, JSS College of pharmacy, Ooty for the technical support.

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