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Synthesis and *in vitro* Biological Evaluation of 6-chloro dibenzo[*c,f*][2,7]naphthyridine

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Abstract

Abstract : A new methodology was found to generate 6-chloro dibenzo[c,f][2,7] naphthyridine derivatives (**6a-f**) which is described from substituted anilines **(1a-f)** and ethyl ethoxymethylenemalonate (**2**) through the intermediates β-anilino-α-carbanilido ethylacrylates **(3a-f)** and 3-carbanilido-4-hydroxyquinolines (**4a-f**). From these intermediates, 6-hydroxy dibenzo[c, f][2, 7]naphthyridines **(5a-f)** were synthesised and converted into the title compounds **(6a-f)**. Antibacterial and cytotoxic activities of some chloronaphthyridines were screened and noted. The structures of the products were established by using IR and NMR techniques.

Keywords: *6-chloro dibenzo[c,f][2,7]naphthyridine, Benzo naphthyridines, Antibacterial activity, Cytotoxic activity.*

1. Introduction

Most of the medicines contain heterocyclic structural framework, among which quinoline finds an important place in the drugs hierarchy. The addition of an extra pyridine ring to the quinoline framework resulting in a benzo fused naphthyridines will be an added advantage. These compounds revealed a broad range of biological activities including antibacterials,[1-3] antimalarials,[4-6] antitumor[7,8] and DNA ligaze inhibitors[9] for the treatment of cancer. Due to broad applications of benzo fused naphthyridine ring system,[10] in our investigation, we focused our interest towards the systems of fused naphthyridines and their derivatives. Hence, it is relevant to mention here the importance and the methods, applied for the synthesis of naphthyridines. As shown in **Fig 1**, among dibenzo[*c,f*][2,7]naphthyridine derivatives **6a**, **6c**, **6d** shows higher antibacterial activity against pathogenic bacteria. Similarly **6e** and **6f** have *in vitro* cytotoxicity against HeLa cell line.

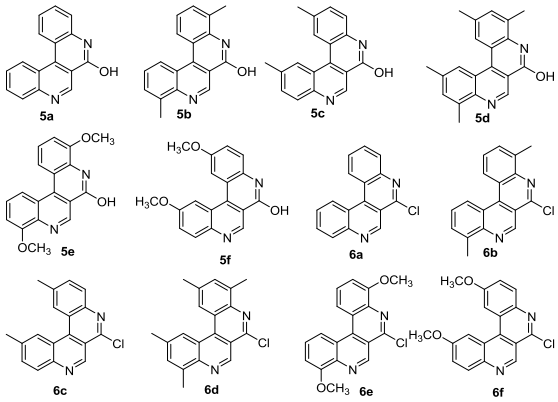

Fig 1. Structures of 6-hydroxy dibenzo[*c, f*][2, 7]naphthyridine and 6-chloro dibenzo[*c,f*][2,7]naphthyridine derivatives.

Recently, the halogenated hetero compounds have gained significant attention due to their remarkable applications in the area of pharmaceuticals. Through this ideal intention, we have to know halogenated heterocycles are the important building block in the synthesis of drugs. Owing to the importance of halogenated heterocycles, we desired to explore the efficient methods for the synthesis of chlorinated heterocycles.

2. Results and Discussion

2.1 Chemistry

The required precursor **4** for the synthesis of chlorinated benzo naphthyridine was synthesised from **3** which in turn was synthesised from **1** with ethyl ethoxymethylenemalonate (**2**) in diphenyl ether at 150 °C.

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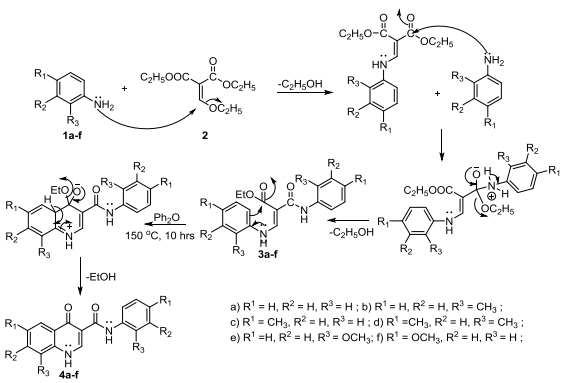
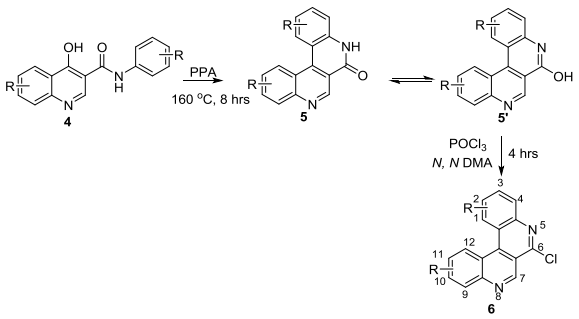
Initially, the reaction started with the compound **1** and ethyl ethoxymethylenemalonate (**2**) which was heated in ethanol at 150 ^{o}C for 2 hrs, there was no change observed in the reaction. After 2 hrs, slight changes appeared in the colour and spot in TLC of the mixture. So the reaction was further optimised with continuation refluxing of the mixture resulting **3** and the yield of 20-30%. Having achieved **3**, the cyclisation was tried in diphenyl ether. The mixture was refluxed in diphenyl ether at 150 ^{o}C for 10 hrs, affording the desired cyclised hydroxyl product **4** in 20-30% yield. Further, we move to cyclise **4** by using versatile cyclising agent PPA after heating the mixture at 160 ^{o}C for 8 hrs.

Now we synthesise **6** from **5** by using simple chlorinating reagent phosphoryl oxy chloride. Hence, the hydroxyl compound **5** was treated with $POCl_3$ in presence of *N, N* – Dimethylaniline as a base for 4 hrs. The obtained desired chlorinated product **6** in 80-95% yield. The reaction was considered as the optimal conditions for the further course of cyclisation reactions.

The probable mechanism for product formation via intermediate is outlined in scheme **1**.



Scheme 1. Proposed mechanism for the formation of precursor 4.



Scheme 2. Different pathways for the synthesis of 6-chloro dibenzo[*c, f*][2, 7] naphthyridine (6) .

2.2. Antibacterial activity

Antibacterial activities of selected compounds **5a**, **5c**, **5d**, **6a**, **6c**, **6d** were screened for their *in vitro* growth inhibitory activity against pathogenic bacteria *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi.* These organisms have multiple antibiotic resistances against a diverse spectrum of commercially available antibiotics. The compounds showed moderate to good results for specified bacteria. They are explained as follows, **6a, 6c** and **6d** showed good antibacterial activity against *S. aeruginosa* with higher zone of inhibition. Rest of the compounds showed only moderate activity. Compound **6a** showed higher antibacterial activity against *Klebsiella pneumonia* with higher zone of inhibition. Rest of the compounds showed only moderate activity. Compound **5c** showed good antibacterial activity against *Escherichia coli* with higher zone of inhibition. Rest of the compounds showed only moderate activity.Compounds **5a**, **5c**, **6a** and **6c** showed better activity against a pathogen *Staphylo coccus* with higher zone of inhibition **5d** and **6d** showed good results at higher concentrations. Rest of the compounds showed only moderate activity.

Compounds **5c**, and **6a** showed good activity against the pathogen *Salmonella typhi* with higher zone of inhibition, whereas **6c** was active only at higher concentrations. Rest of the ompounds showed only moderate activity.

2.3 Biomolecular interaction and cytotoxic studies

2.3.1 DNA binding-Absorption titration

The electronic absorption spectra of compounds displayed absorption bands in the range of 230 to 600 nm out of which the high energy absorption bands appeared due to the type $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$.[11] Electronic absorption spectra of compounds without as well as with the added CT DNA are shown in **Fig. 2.** From the electronic absorption spectral data, it is clear that upon increasing concentration of DNA added to the quinoline compounds, absorption bands showed hypochromism. The nucleotide binding activity of newly synthesized angular dibenzo fused naphthyridine derivatives has been studied. It was found that they have definite interaction with DNA. Further, binding efficiency was also measured by percentage hypochromism exhibited by them on addition of DNA. The percentage hypochromism exhibited by the compounds as 45, 39, 36, 31, 25 and 18 % revealed the binding activity decrease in the following order **6f**, **5f**, **6c**, **6a**,**5c** and **5d.**

2.3.2 Cytotoxicity Studies

The survey of quinoline based anticancer compounds endowed us to screen the antitumor activity of hydroxyl and chloro substituted [2,7] naphthyridines against the HeLa cell line and IC_{50}

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values were calculated in micromillilitre (µg/mL). **5c**, **6c** and **6f** compounds showed moderate toxicity to the cancer cell lines. **6f** showed much more activity than the compounds **5c** and **6c**. On the other hand, by replacing hydroxyl group (**5c**) with chloro group (**6c** and **6f**), cytotoxicity was increased against HeLa cell line. The methoxy derivative showed better activity when compared with methyl derivative (**6c**).

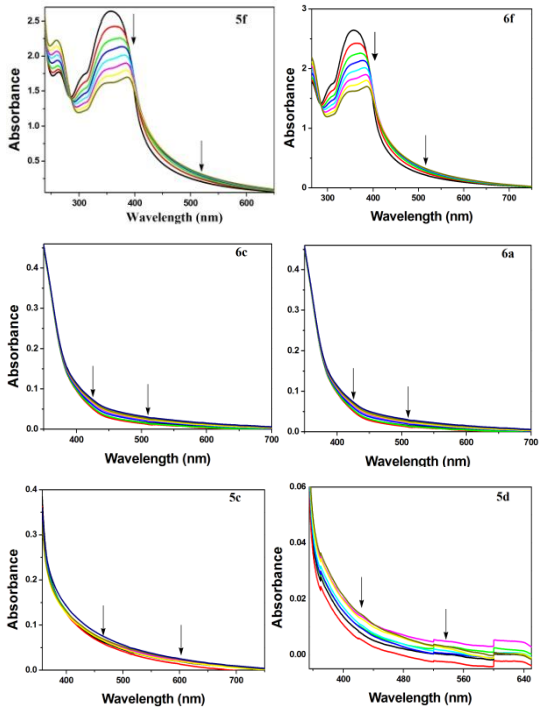

Fig. 2 Changes in the electronic absorption spectra of the compounds (15 µM) with increasing concentrations of CT-DNA (45 µM).

Table 1

Compound	Cytotoxic activity (IC_{50}, µg/mL)
5c	52.4
6c	65.1
6f	41.2

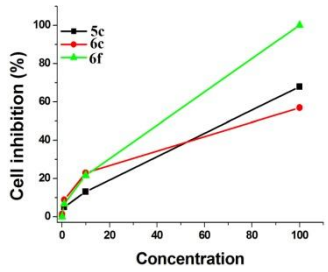

Fig 3. Cell survival curves of HeLa cell line upon treating with compounds

3. Conclusion

In the first step to achieve angular chlorinated naphthyridines (**6a-6f**), two aromatic amines were substituted in ethoxymethylenemalonate (**2**) by the removal of ethanol. From this intermediate 3 various cyclisation processes were carried out using the reagents diphenyl ether and poly phosphoricacid to get hydroxyl naphthyridines (**5a-f**). Finally hydroxyl derivatives were chlorinated by $POCl_3$. In the biological activities, it is noted that the chloro naphthyridines generally showed better results compared with hydroxy naphthyridines. Chloro naphthyridines displayed good antibacterial activity on increasing their concentrations. The methoxy and chloro derivatives exhibit better cytotoxic activity when compared with methyl and hydroxyl derivatives against HeLa cell line. This lead us framing halogenated naphthyridine analogs to increase the *in vitro* biological activities.

4. Experimental

Melting points were determined on Boetieus Micro heating table apparatus and are uncorrected. They are expressed in degree centigrade (^oC). The IR spectra were recorded on Perkin Elmer 537 spectrophotometer or Shimadzu-8201 FT instrument, using KBr disc or Nujol mull and the absorption frequencies are expressed in reciprocal centimeters (cm^{-1}). Microanalyses were performed on vario EL-III elemental analyser (GmbH) CHN analyzer. The ^1H and ^{13}C NMR spectra were recorded on AMX-400 (400 MHz) spectrophotometer, using tetramethylsilane (TMS) as an internal reference. The chemical shifts are quoted in parts per million (ppm).Mass spectra were recorded on JEOL jms-D 300 (70 eV) and EI-MS mass spectrometer.

4.1. Preparation of β-anilino-α-carbanilido acrylate (3): general procedure

Aniline 0.1098 moL and ethoxy methylenemalonate 0.0549 moL were stirred at 150 ^oC for 4 hrs and ethanol was distilled as it was being generated. Then the reaction mixture was cooled and washed with 15 mL of ethyl acetate. The resulting mixture was column chromatographed with petroleum ether/ ethyl acetate (99:1) (v/v) as eluent giving the product as white crystals.

4.1.1. β-anilino-*α*-carbanilido ethylacrylate (**3a**)

Yield (30 %); mp: 114 - 115 ºC; IR (KBr, υ_{max}) cm^{-1} : 1716 (-C=O ester), 2978 (-CH), 1642 (-NHCO), 1589 (-C=N), 1257 (-O-CH_2-), 3179, 3234 (two -NH); CHN analysis (%): Calcd. C 69.65, H 5.84, N 9.02; ($C_{18}H_{18}N_2O_3$) (310.385) Found: C 69.69, H 5.80, N 9.07.

4.1.2. β-(o-toludino)-α-(2-methylcarbanilido) ethyl*acrylate (**3b**)*

Yield (31 %); mp: 132 - 134 ºC; IR (KBr, υ_{max}) cm^{-1} : 1714 (-C=O ester), 2980 (-CH), 1658 (-NHCO), 1586 (-C=N), 1261 (-O-CH_2), 3176, 3231 (two -NH); CHN analysis (%): Calcd. C 70.98, H 6.55, N 8.27; ($C_{20}H_{22}N_2O_3$) (338.407) Found: C 70.93, H 6.57, N 8.30.

4.1.3. β-(p-toludino) -α-(4-methylcarbanilido) ethyl*acrylate (**3c**)*

Yield (20 %); mp:164 -166 ºC; IR (KBr, υ_{max}) cm^{-1}: 1710 (-C=O ester), 2985 (-CH), 1665 (-NH**CO**), 1605 (-C=N), 1254 (-O-CH_2), 3177, 3229 (two -NH); ^{1}H NMR ($CDCl_3$)δppm : 2.3 (s, 6H, C_4–CH_3, C'_4 -CH_3), 1.38-1.4 (t, 3H,**CH_3** – CH_2-O), 4.3 (q, 2H, -**CH_2**-O), 7.15 (d, 2H, C_2-H, C_6-H, *J* = 8 Hz), 7.21 (d, 2H, C_3-H & C_5-H, *J* = 8 Hz), 7.5 (d, 2H, C'_2 & C'_6, *J*=7 *Hz*,), 7.0 (d, 2H, C'_3-H & C'_5-H *J* = 8.5 *Hz*,), 8.5 (d, 1H, methine proton, *J* = 13.5 Hz), 10.90 (1H, s, -CO-**NH**), 12.34 (d,1H, -CH-**NH**); CHN analysis (%): Calcd. C 70.98, H 6.55, N 8.27; ($C_{20}H_{22}N_2O_3$) (338.407) Found: C 70.92, H 6.52, N 8.30.

4.1.4. β-(2, 4-dimethylanilino)-α-(2, 4-dimethylcarbanilido) ethyl*acrylate (**3d**)*

Yield (20 %); mp: 108 -109 ºC; IR (KBr, υ_{max}) cm^{-1} : 1718 (-C=O ester), 2980 (-CH), 1658 (-NHCO), 1501(-C=N), 1252 (-O-CH_2), 3169, 3235 (two -NH); CHN analysis (%): Calcd. C 72.10, H 7.15, N 7.64; ($C_{22}H_{26}N_2O_3$) (366.461) Found: C 72.11, H 7.62, N 6.60.

4.1.5. β-(o-anisido)-α-(2-methoxycarbanilido) ethyl*acrylate (**3e**)*

Yield (24 %); mp: 146 -147 ºC; IR (KBr, υ_{max}) cm^{-1}; 1708 (-C=O ester), 2980 (-CH), 1529 (-C=N), 1642 (-NHCO), 1261 (-O-CH_2), 3184, 3245 (two -NH); CHN analysis (%): Calcd. C 64.85, H 5.98, N 7.56; ($C_{20}H_{22}N_2O_5$) (370.405) Found: C 64.89, H 5.93, N 7.52.

4.1.6. β-(p-anisido)-α-(4-methoxycarbanilido) ethyl*acrylate (**3f**)*

Yield (26.9 %); mp: 132 - 134 ºC; IR (KBr, υ_{max}) cm^{-1}: 1711 (-C=O ester), 2972 (-CH), 1525 (-C=N), 1646 (-NHCO), 1253 (-O-CH_2-), 3189, 3243 (two -NH); CHN analysis (%): Calcd. C 64.85, H 5.98, N 7.56; ($C_{20}H_{22}N_2O_5$) (370.405) Found: C 64.89, H 5.92, N 7.56.

*4.2. Preparation of 3-carbanilido-4-hydroxy quinoline -(**4**): general procedure*

β-anilino-*α*-carbanilido acrylate (**2**) was refluxed in diphenylether (150 mL) at 150 ^{o}C for 10 hrs. Then the resulting mixture was washed three times with 10 mL of petroleum ether and the mixture was column chromatographed over silica gel using petroleum ether: ethyl acetate (20: 80) mixture as eluent.

*4.2.1. 3-carbanilido-4-hydroxy quinoline (**4a**)*

Yield (20 %); mp: >300 ºC; IR (KBr, υ_{max}) cm^{-1}: 1665 (-NHCO), 1648 (-NHCO), 3436 (-NH), 1561 (-C=N); CHN analysis (%): Calcd. C 72.73, H 4.57, N 10.60; ($C_{16}H_{12}N_2O_2$) (264.197) Found: C 72.78, H 4.55, N 10.64.

*4.2.2. 3-(2'-methylcarbanilido)-4-hydroxy-8-methyl quinoline (**4b**)*

Yield (23 %); mp: >300 ºC; IR (KBr, υ_{max}) cm^{-1}: 1684 (-NHCO), 1639 (-NHCO), 3246 (-NH), 1537 (-C=N); CHN analysis (%): Calcd. C 73.95, H 5.51, N 9.58; ($C_{18}H_{16}N_2O_2$) (292.338) Found: C 73.94, H 5.57, N 9.56.

*4.2.3. 3-(4'-methylcarbanilido)-4-hydroxy-6-methyl quinoline (**4c**)*

Yield (22 %); mp: 300 ºC; IR (KBr, υ_{max}) cm^{-1}: 1663 (-NHCO), 1631(-NHCO), 1554 (-C=N), 3302 (-NH); ^{1}H NMR (DMSO) δ ppm: 2.24 (s, 3H, C_6-CH_3), 3.32 (s, 3H, C'_4- CH_3), 8.5 (1H , s, C_2-H), 8.36 (s, 1H, C_5-H), 8.15(1H, d, C_8-H, *J* = 7.5 Hz), 7.3 (d, 2H, C'_2-H & C'_6-H, *J*= 8.5 Hz), 7.0 (s, 1H, C_7-H), 6.8 (d, 2H, C'_3-H, C'_5-H, *J* = 8.5 Hz), 12.3 (1H, s, C_4-OH), 10.15 (bs, 1H, NH); CHN analysis (%): Calcd. C 73.95, H 5.51, N 9.58; ($C_{18}H_{16}N_2O_2$) (292.33) Found: C 73.91, H 5.59, N 9.52.

*4.2.4. 3-(2',4'-dimethylcarbanilido)-4-hydroxy-6,8-dimethyl quinoline (**4d**)*

Yield (30 %); mp: >300 ºC; IR (KBr, υ_{max}) cm^{-1}: 1666 (-NH**CO**), 1639 (-NH**CO**), 3303 (-NH), 1554 (-C=N); ^{1}H NMR (DMSO) δppm : 2.2 (s, 3H, C_6-CH_3), 2.3 (s, 3H, C'_4-CH_3), 2.4 (s, 3H, C_8-CH_3), 2.6 (s, 3H, C'_2-CH_3), 7.0 (d, 1H, C'_5-CH_3, *J* =8 Hz), 7.07(s, 1H, C'_3-H), 7.5 (s, 1H, C_5-H), 8.0 (s, 1H, C_7-H), 8.2 (d, 1H, C'_6-H), 8.7 (d, 1H, C_2-H), 12.1 (d, 1H, NH), 12.3 (s, 1H, OH); CHN analysis (%): Calcd. C 74.97, H 6.29, N 8.74; ($C_{20}H_{20}N_2O_2$) (320.392) Found: C 74.91, H 6.26, N 8.79.

*4.2.5. 3-(2'-methoxycarbanilido)-4-hydroxy-8-methoxy quinoline (**4e**)*

Yield (22 %); mp: >300 ºC; IR (KBr, υ_{max}) cm^{-1}: 1663 (-NHCO), 1629 (-NHCO), 3324 (-NH), 1529 (-C=N), 1260 (-OCH_3); CHN analysis (%): Calcd. C 66.65, H 4.97, N 8.63; ($C_{18}H_{16}N_2O_4$) (324.336) Found: C 66.61, H 4.90, N 8.69.

*4.2.6. 3-(4'-methoxycarbanilido)-4-hydroxy-6-methoxy quinoline (**4f**)*

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Yield (21 %); mp: >300 ºC; IR (KBr, υ_{max}) cm^{-1}: 1662 (-NHCO), 1635 (-NHCO), 1556 (-C=N), 3301 (-NH), 1239 (-OCH_3); CHN analysis (%): Calcd. C 66.65, H 4.97, N 8.63; ($C_{18}H_{16}N_2O_4$) (324.336) Found: C 66.51, H 4.82, N 8.49.

*4.3 Preparation of 6-hydroxy dibenzo[c, f][2, 7]naphthyridine (**5**): general procedure*

3-carbanilido-4-hydroxy quinoline (**3**) was added to the activated PPA (10 mL) [P_2O_5 (6.4 g) + H_3PO_4 (3.6 mL)]. The whole of the mixture was heated at 160 ^oC for 8 hrs. After the completion of reaction, the reaction mixture was poured into the crushed ice and then the precipitated solid filtered and dried (mp above 300 ^oC).

*4.3.1. 6-hydroxy dibenzo[c, f][2, 7]naphthyridine (**5a**)*

Yield (24 %); mp: >300 ºC; IR (KBr) γ_{max} cm^{-1}: 1668 (-NHCO), 1600 (-C=N), 3089 (-NH); ^1H NMR (DMSO-d_6) δppm; 7.9-8.4 (m, 8H, Ar-H), 8.5 (s, 1H, C_7-H), 14.2 (s, 1H, C_6-OH); CHN analysis (%): Calcd. C 78.03, H 4.09, N 11.30; ($C_{16}H_{10}N_2O$) (246.08) Found: C 77.87, H 4.00, N 11.19.

*4.3.2. 6-hydroxy-4, 9-dimethyl dibenzo[c, f][2, 7]naphthyridine (**5b**)*

Yield (24 %); mp: >300 ºC; IR (KBr, υ_{max}) cm^{-1}: 1662 (-NHCO), 1572 (-C=N), 3324 (-NH), 1260 (-OCH_3); ^1H NMR (DMSO-d_6) δ ppm: 2.4 (s, 6H, C_4-CH_3, C_9-CH_3), 8.0-8.4 (m, 6H, C_1-H, C_2-H, C_3-H, C_{10}-H, C_{11}-H, C_{12}-H) 8.3 (s, 1H, C_7-H), 13.5 (s, 1H, C_6-OH); CHN analysis (%): Calcd. C 78.81, H 3.77, N 10.21; ($C_{18}H_{14}N_2O$) (274.323) Found:C 78.84, H 3.70, N 10.27.

*4.3.2. 6-hydroxy-2, 11-dimethyl dibenzo[c, f][2, 7]naphthyridine (**5c**)*

Yield (28 %); mp: >300 ºC; IR (KBr, υ_{max}) cm^{-1}: 1673 (-NHCO), 1584 (-C=N), 3225 (-NH); ^1H NMR (DMSO-d_6) δ ppm: 1.9 (s, 6H, C_2-CH_3, C_{11}-CH_3), 8.1 (s, 1H, C_1-H),8.0 (d, 2H, C_3-H, C_{10}-H, *J*=8 Hz), 8.5 (d, 2H, C_4-H, C_9-H, *J*=7.5 Hz), 8.8 (s, 1H, C_7-H), 7.7 (s, 1H, C_{12}-H), 15.5 (s, 1H, C_6-OH); Mass (EI^+): M^+ 274; CHN analysis (%): Calcd. C 78.81, H 3.77, N 10.21; ($C_{18}H_{14}N_2O$) (274.323) Found: C 78.74, H 3.71, N 10.13.

*4.3.2. 6-hydroxy-2,4,9,11-tetramethyl dibenzo[c, f][2, 7]naphthyridine (**5d**)*

Yield (30 %); mp: >300 ºC; IR (KBr, υ_{max}) cm^{-1} : 1694 (-NH**CO**), 1578 (-C=N), 3174 (-NH); ^1H NMR (DMSO-d_6) δ ppm : 1.8 (s, 6H, C_2-CH_3, C_{11}-CH_3), 2.5 (s, 6H, C_4-CH_3, C_9-CH_3), 7.0 (s, 1H, C_1-H), 7.2 (s, 1H, C_{12}-H), 7.8 (s, 2H, C_3-H, C_{10}-H), 8.7 (s, 1H, C_7-H), 13.5 (s, 1H, C_6-OH); CHN analysis (%): Calcd. C 79.47, H 6.00, N 9.26; ($C_{20}H_{18}N_2O_2$) (302.247) Found: C 79.35, H 5.88, N 9.15.

*4.3.2. 6-hydroxy-4, 9-dimethoxy dibenzo[c, f][2,7]naphthyridine (**5e**)*

Yield (22 %); mp: >300 ºC; IR (KBr, υ_{max}) cm^{-1}: 1673 (-NHCO), 1566 (-C=N), 3046 (-NH); ^1H NMR (DMSO-d_6) δ ppm: 3.8 (s, 6H, C_4-OCH_3, C_9-OCH_3), 8.2-8.5 (m, 6H, C_1-H, C_2-H, C_3-H, C_{10}-H, C_{11}-H, C_{12}-H), 8.7 (s, 1H, C_7-H), 15.2 (s, 1H, C_6-OH); CHN analysis (%): Calcd. C 70.57, H 4.60, N 9.14; ($C_{18}H_{14}N_2O_3$) (306.321) Found: C 70.41, H 4.49, N 9.05.

*4.3.2. 6-hydroxy-2, 11-dimethoxy dibenzo[c, f][2,7]naphthyridine (**5f**)*

Yield (22 %); mp: >300 ºC; IR (KBr, υ_{max}) cm^{-1}: 1673 (-NHCO), 1550 (-C=N), 3353 (-NH); ^1H NMR (DMSO-d_6) δ ppm: 3.5 (6H, s, C_2-OCH_3, C_{11}-OCH_3), 7.9-8.2 (m, 4H, C_3-H, C_4-H, C_9-H, C_{10}-H), 8.3 (s, 1H, C_1-H), 8.5 (s, 1H, C_{12}-H), 8.7 (s, 1H, C_7-H), 15.3 (s, 1H, C_6-OH); CHN analysis (%): Calcd. C 70.57, H 4.60, N 9.14; ($C_{18}H_{14}N_2O_3$) (306.321) Found: C 70.42, H 4.47, N 9.02

*4.4 Preparation of 6-chloro dibenzo[c, f][2, 7]naphthyridine (**6**): general procedure*

6-hydroxy dibenzo[*c, f*][2, 7]naphthyridine (**4**) (0.00182 moL), phosphorous oxy chloride 3 mL (0.408 moL) and 2 drops of N,N-dimethylaniline were taken in a round bottem flask and heated on a water bath for 4 hrs. After completion of the reaction, the reaction mixture was poured into crushed ice and stirred well. The formed precipitate was filtered, washed with water and dried. The chloro compound was purified by coloumn chromatography using petroleum ether : ethyl acetate as eluent (96 : 4).

*4.4.1. 6-chloro dibenzo[c, f][2, 7]naphthyridine (**6a**)*

Yield (84 %); mp: >300 ºC; IR (KBr, υ_{max}) cm^{-1}: 1589 (-C=N), 1042(-C-Cl); ^1H NMR (DMSO-d_6) δ ppm: 7.8-8.3 (m, 8H, Ar-H), 8.6 (s, 1H, C_7-H); CHN analysis (%): Calcd. C 72.60, H 3.43, N 10.58; ($C_{16}H_9ClN_2$) (264.71) Found: C 72.17, H 3.18, N 10.39.

*4.4.2. 6-chloro-4, 9-dimethyl dibenzo[c, f][2, 7]naphthyridine (**6b**)*

Yield (94 %); mp: >300 ºC; IR (KBr, υ_{max}) cm^{-1}: 1586 (-C=N), 1038(-C-Cl); ^1H NMR (DMSO-d_6) δ ppm: 2.5 (s, 6H, C_4-CH_3, C_9-CH_3), 8.1-8.4 (m, 6H, C_1-H, C_2-H, C_3-H, C_{10}-H, C_{11}-H, C_{12}-H), 8.6 (s, 1H, C_7-H); CHN analysis (%): Calcd. C 73.85, H 4.48, N 9.57; ($C_{18}H_{13}ClN_2$) (292.76) Found: C 73.74, H 4.30, N 9.27.

*4.4.3. 6-chloro-2, 11-dimethyl dibenzo[c, f][2, 7]naphthyridine (**6c**)*

Yield (95 %); mp: >300 ºC; IR (KBr, υ_{max}) cm^{-1}: 1584 (-C=N), 1032(-C-Cl); ^1H NMR (DMSO-d_6) δ ppm: 2.2 (s, 6H, C_2-CH_3, C_{11}-CH_3); 8.0 (s, 1H, C_1-H), 8.2 (d, 2H, C_3-H, C_{10}-H, *J*=8 Hz), 8.5 (d, 2H, C_4-H, C_9-H, *J*=7.5 Hz); 8.7 (s, 1H, C_7-H); 7.8 (s, 1H, C_{12}-H); CHN analysis (%): Calcd. C 73.85, H 4.48, N 9.57; ($C_{18}H_{13}N_2Cl$) (292.76) Found: C 73.81, H 4.40, N 9.28.

*4.4.4. 6-chloro-2,4,9,11-tetramethyl dibenzo[c, f][2, 7]naphthyridine (**6d**)*

Yield (80 %); mp: >300 ºC; IR (KBr, υ_{max}) cm^{-1} : 1578 (-C=N), 1052 (-C-Cl) ^1H NMR (DMSO-d_6) : 2.3 (s, 6H, C_2-CH_3, C_{11}-CH_3), 2.5 (s, 6H, C_4-CH_3 C_9-CH_3), 7.2 (s, 1H, C_1-H), 7.4 (s, 1H, C_{12}-H), 7.9 (s, 2H, C_3-H, C_{10}-H), 8.9 (s, 1H, C_7-H); CHN analysis (%): Calcd. C 74.88, H 5.34, N 8.73; ($C_{20}H_{17}ClN_2$) (320.82) Found C 74.65, H 5.23, N 8.65.

*4.4.5. 6-chloro-4, 9-dimethoxy dibenzo[c, f][2,7]naphthyridine (**6e**)*

Yield (82 %); mp: >300 ºC; IR (KBr, υ_{max}) cm^{-1}: 1574 (-C=N), 1027 (-C-Cl); ^1H NMR (DMSO-d_6) δ ppm: 3.6 (s, 6H, C_4-OCH_3, C_9-OCH_3), 8.2-8.6 (m, 6H, C_1-H, C_2-H, C_3-H, C_{10}-H, C_{11}-H, C_{12}-H), 8.7 (s, 1H, C_7-H); CHN analysis (%): Calcd. C 66.57, H 4.03, N 8.63; ($C_{18}H_{13}ClN_2O_2$) (324.76) Found C 66.31, H 3.89, N 8.55.

*4.4.6. 6-chloro-2, 11-dimethoxy dibenzo[c, f][2,7]naphthyridine (**6f**)*

Yield (80 %); mp: >300 ºC; IR (KBr, υ_{max}) cm^{-1}: 1579 (-C=N), 1033(-C-Cl); ^1H NMR (DMSO-d_6) δ ppm: 3.4 (6H, s, C_2-OCH_3, C_{11}-OCH_3), 7.8-8.2 (m, 4H,C_3-H, C_4-H, C_9-H, C_{10}-H), 8.3 (s, 1H, C_1-H), 8.5 (s, 1H, C_{12}-H), 8.6 (s, 1H, C_7-H); CHN analysis (%): Calcd. C 66.57, H 4.03, N 8.63; ($C_{18}H_{13}ClN_2O_2$) (324.76) Found: C 66.37, H 3.93, N 8.43.

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6. References

[1] A.Gangjee, J. Shi and S.F. Queener, Synthesis and Biological Activities of Conformationally Restricted, Tricyclic Nonclassical Antifolates as Inhibitors of Dihydrofolate Reductase *J. Med. Chem.* **40**, 1930. (1997)

[2] Y. Zhang, B. Illarionov, A. Bacher, M. Fischer, G.I. Georg, Q. Ye and D. Velde, Synthesis and Enzyme Inhibitory Activity of the *S*-Nucleoside Analogue of the Ribitylaminopyrimidine Substrate of Lumazine Synthase and Product of Riboflavin Synthase *J.Org.Chem.*, 72, 2769, (2007).

[3] E. Laxminarayana, T. Karunakar, S. S. Shankar, and M.T. Chary, A study on antibacterial activity of substituted1, 8-naphthyridines containing carbaldehydes, methyleden hydrazines, thiadiazolamines and triazolethiols*AdvancedDrug Delivery Reviews*, **2**, 6, (2012).

[4] K. Gorlitzer, C. H. Enge, P. G. Jones, H. Jomaa, J. Wiesner, *Pharmazie*, Benzo[c][2,7]naphthyridine-5-yl-amines and benzo[h][1,6]naphthyridine-5-yl-amines--potential antimalarials, 62**,** 89, (2007).

[5] S. Olepu and P. K. Suryadevara, 2-Oxo-tetrahydro-1, 8-naphthyridines as selective inhibitors of malarial protein farnesyltransferaseand as anti-malarials *Bioorganic and Medicinal Chemistry Letters*, **18**, 494, (2008)

[6] D. J. Sullivan, Theories on malarial pigment formation and quinoline action, *Int J Parasitol*, 32, 1645, (2002).

[7] Fadda, A. M. El-Defrawy, and S. A. El-Habiby, Synthesis, cytotoxicity evaluation, DFT molecular modeling studies and quantitative structure activity relationship of novel 1, 8-naphthyridines, *The American Journal of Organic Chemistry*, **2**, 87 (2012).

[8] M. Farag, A. S. Mayhoub, S. E. Barakat, and A. H. Bayomi, Synthesis of new N-phenylpyrazole derivatives with potent antimicrobial activity, *Bioorganic and Medicinal Chemistry***, 16,** 881, (2008).

[9] J. P. Surivet, R. Lange, C. Hubschwerlen, W. Keck, J. L. Specklin, D. Ritz, D. Bur, H. Locher, P.Seiler, D. S Strasser, L. Prade, C. Kohl, C. Schmitt, G. Chapoux, E. Ilhan, N. Ekambaram, A. Athanasiou, A. Knezevic, D. Sabato, A. Chambovey, M. Gaertner, M. Enderlin, M. Boehme, V. Sippel and P. Wyss, Structure-guided design, synthesis and biological evaluation of novel DNA ligase inhibitors with in vitro and in vivo anti-staphylococcal activity, *Bioorg. Med. Chem. Lett.*, **22**, 6705, (2012).

[10] A. Murugesh, K. Poongodi and S.P. Rajendran, Synthesis and biological evaluation of dibenzo angular fused naphthyridines, *Biologically Active Molecules*, **96**, 364, (2012).

[11] E. C. Long and J. K. Barton, On demonstrating DNA intercalation, *Acc. Chem. Res.*, 23, 271, (1990).