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Synthesis and Biological Evaluation of some 5-Aza-indeno[1,2-b]fluorine derivatives as Anticancer agents

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Abstract

Reaction of 1-indanone with aromatic aldehydes in presence of ammonium acetate afforded the corresponding 6,12-Dihydro-5-aza-indeno[1,2-b]fluorine **1-8** in excellent yields.The structures of the prepared compounds were confirmed by elementary microanalyses and substantiated with IR, NMR and X-ray spectral data. Biological screening of the prepared compounds revealed significant anticancer activity of some of derivatives.

Keywords: *Indeno[1,2-b]fluorines*, X-ray crystallography, Anticancer activity.

1.Introduction

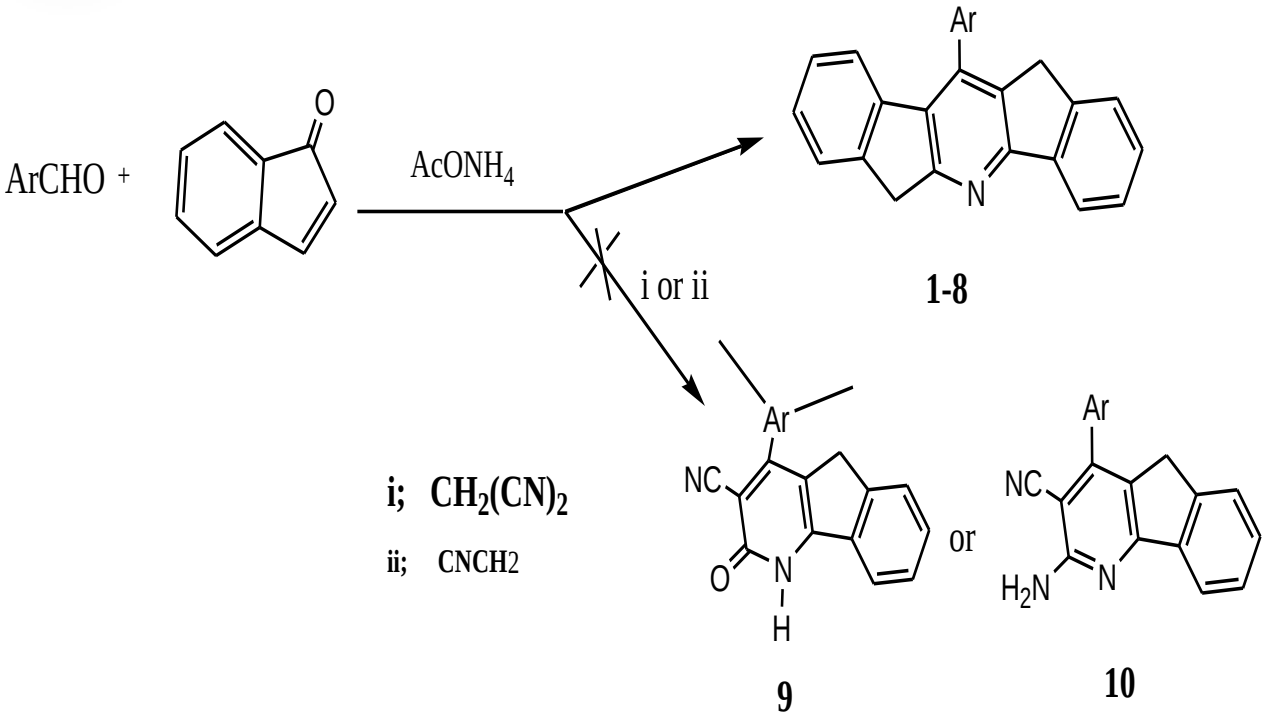
Fluorene and its derivatives also have different types of biological activities, such as anti-inflammatory,[1] antitumour activites,[2,3] antifungel,[4] antimicrobial,[4,6] DNA damaging[7,8] and

cytotoxic[9] activities. Azafluorenone derivatives have been reported to possess activities of aldose reductase inhibition[10] and cytotoxic activity.[11,12] 6,8-Dihydroxy-7-methoxy-1- methyl-azafluorenone compound exhibited potent cytotoxic activities.[13] However, Wilson, DeEds, and Cox,[14,15] reported that 2-acetylaminofluorene is a highly active carcinogen compound that attacks numerous sites in the rat and mouse. In addition, the parent hydrocarbon, fluorene, and several of its other derivatives have been tested for carcinogenic activity in the rat. Fluorene and its oxidation product, fluorenone, are inactive.[16] While 2-nitrofluorene is only weakly carcinogenic[17] its reduction product, 2-aminofluorene, is highly active although somewhat less so than 2-acetylaminofluorene.[18-22] All these versatile properties of this class of hydrocarbons encouraged us to synthesise some new products from this family with cytotoxic activity. Here we report the synthesis and crystal structures of three new molecules along with their theoretical studies.

2. Results and Discussion

2.1*Chemistry*

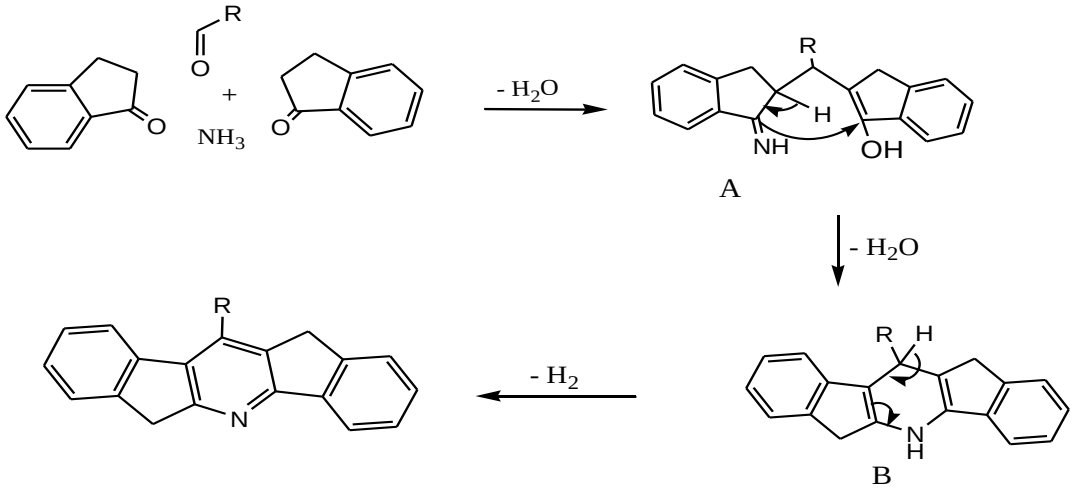
In 1999 Katritzky *et al*.[23] reported a [3+2+1] synthesis of Indeno[1,2-b]pyridine derivatives in good yield from the reaction of 1-indanone and the appropriate α,β-unsaturated ketone in precence of ammonium acetate. In more recent work Rong *et al*.[24] found an efficient and convenient multicomponent reaction for the preparation of 4-aryl-2-oxo-2,5-dihydro-1H-indeno[1,2-b]pyridine-3-carbonitrile derivatives by the reaction of 2,3-dihydroinden-1-one, aromatic aldehydes, and malononitrile in the presence of sodium hydroxide under solvent-free condition is reported. Moreover, Al-Mutairi *et al*,[25] isolated the 5,6-dihydrobenzo[h]quinoline derivatives by condensation of the corresponding 2-arylidene-1-tetralone with malononitrile in the presence of ammonium acetate or via one-pot multicomponent reactions (MCRs) of aldehydes, malononitrile, 1-tetralone and ammonium acetate. Recently,[6] we found that the one-pot multicomponent reaction (MCR) of the 3,4-dihydro-2H-naphthalene-1-one**,** aromatic aldehydes, an excess of ammonium acetate and ethyl cyanoacetate in boiling ethanol afforded the target 3-Cyano-2-oxo-4-aryl-1,2,5,6-tetrahydrobenzo[h]quinolines. Motivated by these results, we investigated a multicomponent reaction of 1-indanone with aromatic aldehydes and malononitrile or ethyl cyanoacetate in presence of ammonium acetate in an attempt to prepare the corresponding 2-amino-4-aryl-5H- indeno[1,2-b]pyridine-3-carbonitrile or 4-aryl-2-oxo-2,5-dihydro-1-indeno[1,2-b]pyridine-3-carbonitrile respectively. However, we found that the reaction yielded the corresponding 6,12-Dihydro-5-aza-indeno[1,2-b]fluorine **1-8** instead of the expected indeno-[1,2-b]pyridine-3-carbonitrile analoges **9** or **10** (Scheme 1). The same products were obtained when we carried the reaction in absence of malononitrile or ethyl cyanoacetate.



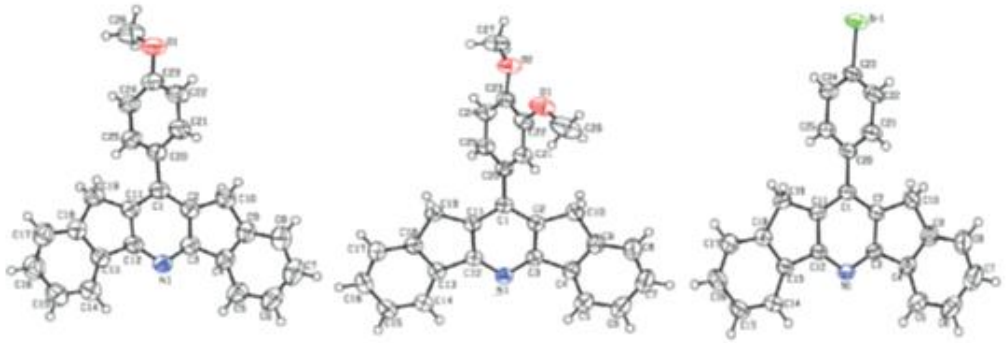
R=4-ClC_6H_4, 4-BrC_6H_4, 4-$CH_3OC_6H_4$, 2-$CH_3OC_6H_4$, 3,4-$(CH_3O)_2C_6H_3$, 4-(OH)-3-$(OCH_3)C_6H_3$, 4-$(CH_3)_2NC_6H_4$, 3-Theinyl

, **Scheme 1**

A possible mechanism of the formation of the 6,12-Dihydro-5-aza-indeno[1,2-b]fluorine derivatives may be explained according to the following scheme:



The ^{1}H-NMR spectra of fluorine derivatives **1-8** exhibited besides the aromatic protons at δ 6.73-7.89 ppm, a singlet of four proton intensity at δ 3.42-3.45 ppm for $2CH_2$.The structures were further supported by ^{13}C-NMR which showed in addition to the expected number of aromatic carbons as well as a signal at 33.71-33.92 for the CH_2 carbon. The conclusive proof of the structure arise from the X-ray crystallography data which confirm the 6,12-Dihydro-5-aza-indeno[1,2-b]- fluorine structure (**Figure I**).



3 **5** **2**

Fig. I. ***X- Ray crystallography of 6,12-Dihydro-5-aza-indeno[1,2-b]fluorine derivatives 2,3 and 5***

2.2. In vitro MTT *cytotoxicity assay*

Five of the prepared 5-aza-indeno[1,2-b]fluorine derivatives**2,3,5,6** and **8** were evaluated for their *in vitro* cytotoxic effect via the standard MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method[26,27] against a panel of three human tumor cell lines namely; Caucasian breast adenocarcinoma MCF7, hepatocellular carcinoma HePG2 and colon carcinoma HT29. The results are presented in Table 1 as LC_{50} (µg/mL) which is the lethal concentration of the compound which cause death of 50% of the cells in 24h. The obtained data revealed that, the three tested human tumor cell lines exhibited variable degree of sensitivity profiles towards the tested compounds. Among these, compounds **3** and **5** showed pronounced activity against the human colon carcinoma HT29 cell line with LC_{50} values 11.5 and 10.1 µg/mL, respectively. Moreover, a remarkable cytotoxic potential was displayed by the two other compounds **2,6** and **7** against the same cell line (18.2 and 15.4 µg/mL). Furthermore, the growth of the human hepatocellular carcinoma HePG2 cell line was found to be moderately inhibited by the above analogs **2,3,5,6** and **8** with LC_{50} values range of 20.2-37.7 µg/mL. Among these, the highest cytotoxic activity was displayed by compound **5** (LC_{50} values 20.2 µg/mL). On the other hand, human breast cancer MCF 7 was proved to be the least sensitive among the cell lines tested as it was affected by only two of the test compounds **3** and **5** (Table **1**). Further interpretation of the results revealed that, compounds **3** and **5** showed considerable broad spectrum of cytotoxic activity against the three tested human tumor cell lines. In particular, compounds **5** proved to be the most active members in this study with a broad spectrum of activity against the tested cell lines, with special effectiveness against the human colon carcinoma HT29 and human breast cancer MCF 7 cell lines (LC_{50} values 10.1 and 20.2 µg/mL, respectively) (Table **1**).

Table 1. Cytotoxic effects LC_{50}; µg/mL[a] of the compounds **2,4,5,6** and **8**

Compd no.	Human colon carcinoma HT29	Human hepatocellular carcinoma HePG2	Human breast cancer MCF 7
2	18.2	37.7	-[b]
3	11.5	25.6	15.7
5	10.1	20.2	8.7
6	15.4	32.2	-
8	19.9	30.8	-
Doxorubicin[c]	21.1	1.69	2.14

[a]LC50: Lethal concentration of the compound which causes death of 50% of cells in 24h (µg/mL).

[b] Totally inactive against this cell line.

[c] positive control cytotoxic agent.

3. Experimental

3.1 *Chemistry*

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu FT-IR 8400S infrared spectrophotometer using the KBr pellet technique. ^{1}H and ^{13}C NMR spectra were recorded on The 1H NMR spectra were recorded on a Varian EM 360 spectrometer; using tetramethylsilane as the internal standard and DMSO-d_6 as a solvent (Chemical shifts in δ, ppm). Splitting patterns were designated as follows: *s*: singlet; *d*: doublet; *m*: multiplet; *q*: quartet. Elemental analyses were performed on a 2400 Perkin Elmer Series 2 analyzer and the found values were within ±0.4% of the theoretical values. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at λ 254.

General procedure for preparation of 6,12-Dihydro-5-aza-indeno[1,2-b]fluorine 1-8

Method A

A mixture of the appropriate aromatic aldehyde (10 mmol), 1-indanone (1.1g, 10 mmol), ethyl cyanoacetate or malononitril (10 mmol) and ammonium acetate (6.2 g, 80 mmol) in absolute ethanol (30 mL) was refluxed for 8 h. The reaction mixture was cooled and the formed precipitate was filtered, washed with water, dried and recrystallized from the appropriate solvent.

Method B

A mixture of the appropriate aromatic aldehyde (10 mmol), 1-indanone (1.1g, 10 mmol), and ammonium acetate (6.2 g, 80 mmol) in absolute ethanol (30 mL) was refluxed for 8 h. The reaction mixture was cooled and the formed precipitate was filtered, washed with water, dried and recrystallized from the appropriate solvent.

1: Rrecrystallized from DMF as needles. (3.3g, 80%) m.p. 273-275°C. ^{1}HNMR (δ/ppm,DMSO-d_6): 3.43 (s,2H,H-12),7.10-7.88 (m,12H,ArH). ^{13}CNMR(δ/ ppm,DMSO-d_6): 33.71(CH_2),123.63,126.12,126,82, 126.94, 127.02, 128.42, 129.15,132.37,136.24,137.25, 140.46,148.25, 157.02(ArC). Anal.% Calcd for $C_{25}H_{16}$Cl N: C,82.07; H, 4.41; N, 3.83. Found: C, 82.12; H, 4.38; N, 3.73.

2: Rrecrystallized from DMF as needles. (3.3g, 80%) m.p. 241-242°C.^{1}HNMR(δ/ppm,DMSO-d_6):3.45(s,4H,2CH_2),7.51-7.83(m,12H, ArH). ^{13}CNMR (δ/ppm, DMSO-d_6):33.74 (CH_2), 116.80, 122.00,123.49,125.50, 127.55, 130.40,131.79,134.17,149.13,158.39 (ArC). Anal.% Calcd for $C_{25}H_{16}$Br N: C,73.18; H, 3.93; N, 3.41. Found: C, 73.21; H, 4.10; N, 3.53.

3: Rrecrystallized from ethanol/DMF as needles. (2.6g, 74%) m.p. 348-350°C. ^{1}HNMR (δ/ppm, DMSO-d_6): 3.74(s,3H,CH_3O), 3.42(s,4H,2CH_2),6.82-7.79(m,12H,ArH) .^{13}CNMR (δ/ppm,DMSO-d_6):56.01(CH_3O),33.92(CH_2),114.63,126.02,126.53,127.04,128.46, 129.74, 130.43,136.22, 137.24, 140.42,148.24,156.56, 162.54 (ArC). Anal.% Calcd for $C_{26}H_{19}$NO: C,86.40; H, 5.30; N, 3.88. Found: C,86.54; H, 5.22; N, 3.71

4: Rrecrystallized from ethanol/DMF as needles. (2.6g, 74%) m.p. 228-230°C. ^{1}HNMR (δ/ppm, DMSO-d_6): 3.70(s,3H,CH_3O), 3.44(s,4H,2CH_2),6.90-7.81(m,11H,ArH),12.14(s,1H,OH).^{13}CNMR (δ/ppm,DMSO-d_6):56.10(CH_3O),33.76(CH_2),113.93,114.62,126.14, 126.54, 127.08,127.35 128.14, 129.19,130.53,136.10,

137.21, 142.45,148.42,156.41,160.12, (ArC). Anal.% Calcd for $C_{26}H_{19}NO$: C,86.40; H, 5.30; N, 3.88. Found: C,86.54; H, 5.22; N, 3.70.

5: Rrecrystallized from ethanol as needles. (2.7g, 70%) m.p. 365-366°C.^{1}HNMR(δ/ppm,DMSO-d_6):3.73(s,3H,CH_3O),3.74(s,3H,CH_3O),3.43(s,4H,2CH_2),6.73-7.87(m,11H,ArH).^{13}CNMRm (δ/ppm,DMSO-d_6):56.00(CH_3O),56.01(CH_3O),33.80(CH_2),113.52,115.61,120.22,126.79, 126.90, 127.02,128.51,129.71, 131.32, 136.31, 140.39,148.15, 148.24,156.52,157.04 (ArC). Anal.% Calcd for $C_{27}H_{21}NO_2$: C,82.84; H, 5.41; N, 3.58. Found: C,82.81; H, 5.25; N, 3.61.

6: Rrecrystallized from ethanol/DMF as needles. (2.7g, 72%) m.p. 267-269°C. ^{1}HNMR (δ/ppm, DMSO-d_6): 3.72(s,3H,CH_3O), 3.46(s,4H,2CH_2),6.98-7.82(m,12H,ArH). ^{13}CNMR(δ/ppm,DMSO-d_6): 56.02(CH_3O), 33.87 (CH_2), 114.61,126.12,126.65,127.14,127.34 128.44, 129.64,130.52,136.20, 137.31, 140.51,148.32,156.23,160.12, 162.14 (ArC). Anal.% Calcd for $C_{26}H_{19}NO_2$: C,82.74; H, 5.07; N, 3.71. Found: C,82.62; H, 5.11; N, 3.70.

7: Rrecrystallized from DMF as needles. (2.6g, 74%) m.p. 283-285°C. ^{1}HNMR (δ/ppm, DMSO-d_6): 2.50 (s,6H,2CH_3), 3.45(s,4H,2CH_2),6.81-7.87(m,12H,ArH) .^{13}CNMR (δ/ppm,DMSO-d_6):56.01(CH_3O),33.84 (CH_2),105.96,112.57,115.66,117.25,121.97,126.77,127.84,129.07,131.23,132.45,147.69,149.26,159.85 (ArC). Anal.% Calcd for $C_{26}H_{19}NO$: C,86.40; H, 5.30; N, 3.88. Found: C,86.26; H, 5.17; N, 3.69.

8: Rrecrystallized from DMF as needles. (3.3g, 80%) m.p. 217-219°C.^{1}HNMR (δ/ppm,DMSO-d_6):3.41(s,4H,2CH_2),7.12-8.01(m,11H,ArH).^{13}CNMR(δ/ ppm, DMSO-d_6): 33.79 (CH_2), 126.21, 126.62,127.12,127.23,127.45, 128.04, 129.12, 130.12,138.21,139.51,143.81,146.12,156.45(ArC). Anal.% Calcd for $C_{23}H_{15}NS$: C,81.87; H, 4.48; N, 4.15. Found: C, 81.78; H, 4.12; N, 4.21.

3.2 Biological activity

Methodology of the In vitro MTT cytotoxicity assay

The synthesized compounds were investigated for their *in vitro* cytotoxic effect *via* the standard [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method (MTT)[26,27] against a panel of three human tumor cell lines namely; Caucasian breast adenocarcinoma MCF7, hepatocellular carcinoma HepG2 and colon carcinoma HT29. The procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Stanford, ME, USA). Cells were batch cultured for 10 days, then seeded at concentration of 10x10^3 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37^{o}C for 24h under 5% CO_2 using a water jacketed carbon dioxide incubator

(Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of the test compounds to give a final concentration of (100 – 50 – 25 – 12.5 – 6.25 – 3.125 – 1.56 – 0.78 µg/mL). DMSO was employed as a vehicle for dissolution of the tested compounds and its final concentration on the cells was less than 0.2%. Cells were suspended in RPMI 1640 medium (for HepG2 and HT29 cell lines) and DMEM (for MCF 7 cell line), 1% antibiotic-antimycotic mixture (10,000 IU/mL penicillin potassium, 10,000 µg/mL streptomycin sulphate and 25 µg/mL amphotericin B), and 1% L-glutamine in 96-well flat bottom microplate at 37°C under 5% CO_2. After 24h of incubation, the medium was aspirated, 40 µL of MTT salt (2.5 µg/mL) were added to each well and incubated for further 4h at 37°C under 5% CO_2. To stop the reaction and dissolve the formed crystals, 200 µL of 10% sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37°C. The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent *t*-test by SPSS 11 program. The results are presented in Tables 1as LC_{50} (µg/mL) which is the lethal concentration of the compound which causes death of 50% of the cells in 24 h.

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