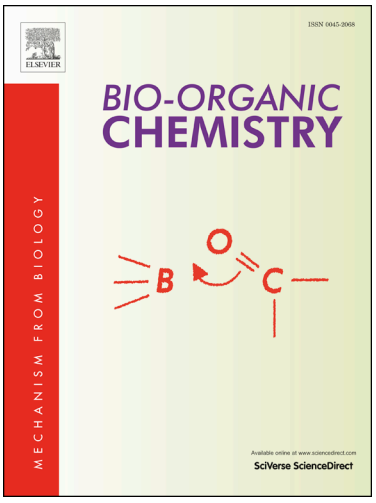

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New Azafluorenones with Cytotoxic and Carbonic Anhydrase Inhibitory Properties: 2-Aryl-4-(4-hydroxyphenyl)-5*H*-indeno[1,2-*b*]pyridin-5-ones

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Abstract

New azafluorenones, 2-aryl-4-(4-hydroxyphenyl)-5*H*-indeno[1,2-*b*]pyridin-5-ones, were prepared to evaluate their cytotoxic/anticancer properties, also their inhibitory effects on hCA I and II isoenzymes. Aryl part was changed as [phenyl (**H1**), 4-methylphenyl (**H2**), 4-methoxyphenyl (**H3**), 4-fluorophenyl (**H4**), 4-bromophenyl (**H5**), 4-chlorophenyl (**H6**), 3-hydroxyphenyl (**H7**), and 4-hydroxyphenyl (**H8**)]. The structure of the synthesized compounds was characterized by ^{1}H-NMR, ^{13}C-NMR and HRMS spectra.

Cytotoxicity results of the series pointed out that the compounds **H6** (PSE: 28.0) and **H5** (PSE: 27.3), with the highest potency selectivity expression (PSE) value, can be considered as leader compounds of the study in designing novel anticancer agents. Additionaly, all azafluorenones synthesized showed a good inhibition profile towards hCA I and II isoenzymes in the range of 54.14-73.72 nM and 67.28-76.15 nM, respectively.

The compounds **H5** and **H6** can be considered for further designs with their cytotoxic and CA inhibitory profiles.

Keywords: Azafluorenone, Phenol, Cytotoxicity, Carbonic Anhydrase

1. Introduction

Cancer is a group of diseases characterized by uncontrolled growth and division of cells which are able to invade other tissues and organs by spreading to other parts of the body through blood and lymph vessels. Despite the advances in the field of anticancer drug discovery, 14.1 million new cases of cancer were diagnosed worldwide in 2012 with 8.2 million deaths. Among the limitations associated with the strategies currently available for cancer treatment, the development of multidrug resistance stands as the major cause of failure as far as chemotherapy is concerned [1].

Phenolic compounds have important biological and pharmacological properties such as antioxidant, antimicrobial, cytotoxic/anticancer, carbonic anhydrase inhibitory, antiobesity, and antidiabetic actions [2-14].

The carbonic anhydrases (CAs) are the metalloenzymes containing zinc ions (Zn^{2+}) and they catalyse the reversible hydration of CO_2 in two-step reaction to yield bicarbonate (HCO_3^-) ion and proton (H^+) [15, 16].

$$CO_2 + H_2O \xleftrightarrow{CA} H_2CO_3 \longleftrightarrow HCO^{-3} + H^+$$

CAs are involved in various biochemical and metabolic processes such as gluconeogenesis, lipogenesis, and ureagenesis [15]. Inhibition of CAs has pharmacologic applications in the field of antiglaucoma, anticonvulsant, anticancer, and anti-infective agents [15].

The CA inhibitory effects of some natural products and their derivatives have been recently investigated and reported that natural compounds carrying phenol or polyphenol moiety had carbonic anhydrase inhibitory activity at micromolar concentrations [17]. The simple phenol molecule was reported to act as an inhibitor of the zinc enzyme CA in a different mechanism of action [18]. Indeed, sulfonamides which are the very well known inhibitors of CA coordinate to

the metal ion from the active site of the enzyme; phenols and derivatives which are less common inhibitor of CA from SO_2NH_2 anchor to the water molecule/hydroxide ion coordinated to the metal ion [19, 20]. In addition, some synthetic compounds having the phenol function showed inhibitory potential on several hCA isoenzymes [3, 4, 13, 14, 21].

The 4-azafluorenone which is an alkaloid from *Annonaceae* species having the chemical structure of 5H-indeno[1,2-b]pyridin-5-one comprises a small but biologically intriguing group of alkaloids [22-24]. In general, they are rigid compounds, as compared to flexible structures, have little conformational entropy and can be more efficiently fitted into the active site of several enzymes [25]. They have the ability to intercalate into the enzyme–DNA complex [26]. Indenopyridines were reported with several biological activities such as anticancer, anti-inflammatory and topoisomerase II inhibitory activities [27-29].

In this study, it was aimed to synthesize new azafluorenone derivatives, 4-(4-hydroxyphenyl)-2-substitutedphenyl-5*H*-indeno[1,2-*b*]pyridin-5-one, to evaluate their cytotoxic/anticancer properties. Substituent on phenyl ring was considered as (**H1**), (**H2**), (**H3**), (**H4**), (**H5**), (**H6**), (**H7**), and (**H8**). It was also planned to investigate their inhibitory effects on hCA I and II isoenzymes to find out new drug candidate/s for further studies.

2. Result and Discussion

2.1. Chemistry

Synthesis of **H1**-**H8** were carried out successfully according to procedure described in detail in the Material and Methods section. The compound synthesized were purified by crystallization from ethanol and the compounds were obtained with the yield of 6.1%-41.1%. The chemical structures of the final compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and HRMS and the data were provided in the Material and Methods section.

2.2. Cytotoxicity

The synthesis of eight new azafluorenone compounds, 4-(4-hydroxyphenyl)-2-substitutedphenyl-5*H*-indeno[1,2-*b*]pyridin-5-one, **H1**-**H8** were reported. Compound **H**, 2-(4-Hydroxybenzylidene)-2*H*-indene-1,3-dione, was used as the starting compound for the synthesis of **H1**-**H8**. Synthetic pathway for **H1**-**H8** was shown in Scheme 1. The chemical structures were elucidated by ^{1}H NMR, ^{13}C NMR, and HRMS spectra. Cytotoxic activities of the compounds were tested towards human oral squamous cell carcinoma cell lines (Ca9-22, HSC-2, HSC-3, HSC-4) and human oral normal mesenchymal cells [gingival fibroblast (HGF), pulp cell (HPC) and periodontal ligament fibroblast (HPLF)]. The cytotoxicity results are presented at Table 1.

The first question to be addressed was whether the compounds synthesized are cytotoxic. The CC_{50} values of the compounds towards cancer cell lines presented at Table 1. They displayed cytotoxic activity at low μM concentrations suggesting that they have antineoplastic property.

The cytotoxicity of the compounds **H1**-**H8** were compared with reference anticancer drug 5-Fluorouracil (5-FU) and melphalan. Most of the compounds were more cytotoxic than the reference compounds 5-FU [**H1** (3.4 times), **H2** (1.5 times), **H3** (1.2 times), **H5** (2.2 times), **H6** (2.2 times), **H7** (1.5 times), **H8** (1.2 times) towards Ca9-22; the compunds **H1** (4.6 times), **H2** ve **H3** (1.2 times), **H5** (2.5 times), **H6** (1.6 times), **H7** (1.7 times), **H8** (1.4 times) towards HSC-2; the compunds **H1** (1.6 times), **H4** (2.7 times), **H5** (1.7 times), **H6** (2.1 times), **H7** (2 times), **H8** (1.3 times) towards HSC-3; **H1** (4.3 times), **H5** (1.6 times), **H6** (1.5 times), **H7** (1.6 times), **H8** (1.4 times) towards HSC-4] and/or melphalane [**H1** (2.6 times), **H2** (1.2 times), **H5** and **H6** (1.7 times), **H7** (1.2 times) towards Ca9-22; the compunds **H1** (3 times), **H5** (1.6 times), **H6** (1 times), **H7** (1.1 times) towards HSC-2; the compunds **H1** (3.9 times), **H5** (1.5 times), **H6** (1.4 times), **H7** (1.5 times), **H8** (1.2 times) towards HSC-4] .

The cytotoxicities of the azafluorenone type compounds (**H1**-**H8**) were always higher than chalcone analogue compound **H** from which they were derived.

Since tumour cells were surrounded by normal cells, it is important for a compound to be a tumour specific cytotoxin. Selectivity index (SI) figures were generated which are the quotients of the average CC_{50} values of the nonmalignant cells and the CC_{50} figure of a compound towards a specific cell line. SI value is a marker of the selectivity of a compound for a spesific cell line. The compound having a SI value over the value of 1 can be considered as tumour selective cytotoxic agent. The SI values of the compounds were presented at Table 1. According to the results at Table 1, many azafluorenone compounds seem tumour specific cytotoxins with the SI value higher than the value of 1 [**H5** (1.5 times), **H6** (1.8 times), **H7** (1.3 times), **H8** (1.5 times) towards Ca9-22; the compouds **H** (3.2 times), **H1** (1.9 times), **H2** (1.8 times), **H3** (2.3 times), **H5** (3.6 times), **H6** (2.8 times), **H7** (3.2 times), **H8** (4.0 times) towards HSC-2; the compounds **H3** (1.2 times), **H2** ve **H4** (1.1 times), **H5** (2.1 times), **H6** (2.9 times), **H7** and **H8** (3.0 times) towards HSC-3; the compound **H8** (3.8 times) towards HSC-4].

The leader compound should possess both remarkable cytotoxic potential and selective cytotoxicity towards tumour cells. Potency selectivity expression (PSE) was devised which is the product of the reciprocal of the average CC_{50} values towards Ca9-22, HSC-2, HSC-3 and HSC-4 cells (a measure of potency) and the average SI figures towards these cell lines (a determination of tumour-selectivity) expressed as a percentage. The PSE value of the compounds were presented at Table1. The PSE values of the compounds **H1-H8** were lower than the 5FU and Melphalan's and higher than the starting compound **H** which is a chalcone derivative from which **H1-H8** were derived. These data suggest that preparation of azafluorenones from chalcone analogue **H** has been a useful chemical modification in terms of cytotoxicity and tumour selectivity.

When PSE values were considered, halogen bearing compounds **H6** (with chlorine) and **H5** (with bromine) had PSE values around 27-28 while fluorine bearing compound **H4** had the value of 2.3 as PSE. It seems that the PSE values of **H5** and **H6** were 10 times higher than H4's.

The position of hydroxy group on the phenyl ring whether it is at meta (**H7**) or para (**H8**) position did not change so much the PSE values of the compounds. Similarly, the type of substituents, whether it is electron donating or attracting, did not affect the PSE values of the compounds in the compounds **H2** (PSE: 7.6) and **H3** (PSE:9.0). But, the replacement of a methoxy (**H3**) group by a hydroxy (**H8**) group in which oxygen has less sterical hidrance led to increase in PSE value about 2.5 times in **H8**. So, substition with chlorine (**H6**) or bromine (**H5**) on phenyl ring, which are halogene, was a useful modification in increasing PSE values comparing with **H1**. However, when another halogene fluorine was considered as a substituent on phenyl ring (**H4**), its substitution was not useful in increasing PSE value. Contrary to the expectation, it decreased PSE value comparing with **H** substition on phenyl (in **H1**) although hydrogen and fluorine have similar size.

So, the cytotoxicity of the compounds may result from the physical properties of the compounds, which direct their kinetics and metabolism in different tissues or differences in the cell lines used, which may have different metabolic characteristics.

2.3. CA Inhibition

CA inhibitory effects of the compounds were tested and the results were presented at Table 2. As shown in Table 2, IC_{50} values were in the range of 54.1–87.7 nM towards hCA I while they were in the range of 10.7–76.153 nM towards hCA II. The IC_{50} values of the reference compound AZA towards hCA I and hCA II were 197.3 nM and 115.5 nM, respectively. All compounds had lower IC_{50} value than AZA toward hCA I and hCA II. When IC_{50} values of the compounds were considered, all compounds had similar selectivity on both hCA I and hCA II isoenzymes. **H3** was 1.3 times more selective towards hCA I than hCA II. According to IC_{50} values of the compounds, methoxy-bearing compound **H3** and hydroxy-bearing compound **H7** were the most effective compounds towards hCA I.

When Ki values of the compounds were considered, Ki values of the compounds were in the range of 4.6577±1.022–8.4757±2.554 nM towards hCA I while Ki values were 3.5061±0.911–10.533±3.197 nM towards hCA II. The Ki values of reference compound AZA were 183.390±19.71 nM and 104.60±27.60 nM towards hCA I and hCA II, respectively.

When Ki values of the compounds were considered, azafluorenone compounds had very low Ki values. All these compounds can be considered as leader of the series towards hCA I. On the other hand **H1** (3.5280±1.135), **H5** (3.8242±0.874) and **H7** (3.5061±0.911) which had the similar and lowest Ki values in series can be considered leader compounds of the series towards hCA II.

When Ki values of the compounds were considered, **H1**, **H3**, **H4**, **H5**, and **H7** were slightly more selective towards hCA II isoenzyme than hCA I since Ki values of the compounds towards hCA II were lower than Ki values of the compounds towards hCA I.

3. Conclusion

Azafluorenone compounds **H1**-**H8** were reported for the first time with detailed spectral analysis. Cytotoxicity experiments pointed out that compound **H6** [4-(4-hydroxyphenyl)-2-(4-chlorophenyl)-5*H*-indeno[1,2-b]pyridin-5-one] and **H5** [4-(4-hydroxyphenyl)-2-(4-bromophenyl)-5*H*-indeno[1,2-b]pyridin-5-one] were leader compounds of the series with the highest PSE values. The compounds **H5** and **H6** can be considered for further designs with their cytotoxic and CA inhibitory profiles.

4. Material and Methods

4.1. Chemistry

All reactions were routinely checked by thin-layer chromatography (TLC) on silica gel 60 F254 (Merck Art 5715) and visualized by UV light or iodine. Melting points (mp) were determined in capillary tubes using an Electrothermal 9100 (IA9100, U.K.) and are uncorrected.

Reagents and solvents were purchased from common commercial suppliers [1,3-indandione (Alfa Aesar), 4-chloroacetophenone, 4-methylacetophenone, 4-hydroxyacetophenone, 3-

hydroxyacetophenone (Fluka), acetophenone, 4-hydroxybenzaldehyde (Merck), 4-methoxy acetophenone, 4-fluoro acetophenone, 4-bromo acetophenone (Aldrich), ammonnium acetate (Riedel-de Haen)] and were used as such. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz (Danbury, U.S.A.) in dimethyl sulfoxide (DMSO). Chemical shifts (δ) are given in ppm, and the spectral data were consistent with the assigned structures. Reactions were carried out in a CEM Discover Microwave Synthesis System, 908010 (Matthews, NC)

4.1.1. 2-(4-Hydroxybenzylidene)-2H-indene-1,3-dione (H), Scheme 1

An aqueous solution of sodium hydroxide (10% w/v, 10 mL) was added into the solution of the 4-hydroxy benzaldehyde (0.02 mol) and 1,3-indandione (0.02 mol) in ethanol (6 mL, Scheme 1). The reaction mixture was stirred at room temperature overnight and poured into water (100 mL) [3, 5, 7-9, 11, 30]. The content was neutralized with hydrochloric acid (10% w/v) to give a yellow solid. It was crystallized from EtOH to yield **H** (43%): m.p: 238-239°C, lit m.p. 238-239°C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ 8.52 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.91-7.90 (m, 4H, Ar-H), 7.74 (s, 1H, vinylic-H), 6.92 ppm (d, 2H, Ar-H, *J* = 8.8 Hz); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 190.7, 189.7, 164.1, 146.9, 142.3, 139.9, 138.3, 136.3, 136.1, 125.8, 125.3, 123.5, 123.4, 116.7 ppm; HRMS (ESI-MS): *m/z* $[M+H]^+$ calcd for $C_{16}H_9O_3$: 249.0552, found: 249.0546.

4.1.2. General method for the preparation of H1-H8, 2-aryl-4-(4-hydroxyphenyl)-5H-indeno[1,2-b]pyridin-5-ones, Scheme 1

A mixture of chalcone analogue **H**, a suitable acetophenone having a substituent at 4 or 3 position of phenyl ring 4-H (**H1**), 4-CH_3 (**H2**), 4-OCH_3 (**H3**), 4-F (**H4**), 4-Br (**H5**), 4-Cl (**H6**), 3-OH (**H7**), 4-OH (**H8**) and anhydrous ammonium acetate in dimethylformamide was heated in a microwave oven (13.8 barr, 120 ^{0}C, 200 W) for 30-60 min for the synthesis of **H1**-**H8** (Scheme 1) (30 min (**H1**, **H5**), 40 min (**H7**, **H8**), 45 min (**H3**, **H4**, **H6**), 60 min (**H2**)). Reactions were

monitored by TLC (chloroform/methanol 4.8:0.2). Whenever the reaction completed, 50 mL of water was added. The solid obtained was filtered, dried and crystallized from ethanol.

4.1.2.1. 4-(4-Hydroxyphenyl)-2-phenyl-5H-indeno[1,2-b]pyridin-5-one (H1), Scheme 1

Yield (6.1%): m.p: 298-299°C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ 10.02 (s, 1H, 4-phenyl 4-OH), 8.25 (dd, 2H, Ar-H, *J* = 8.1, 2.2 Hz), 7.91 (d, 1H, Ar-H, *J* = 7.3 Hz), 7.74 (s, 1H, indenopiridine H-3), 7.69-7.49 (m, 8H, Ar-H), 6.87 ppm (d, 2H, Ar-H, *J* = 8.4 Hz); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 191.1, 166.4, 160.4, 159.8, 149.9, 142.7, 138.2, 135.9, 135.6, 132.1, 131.9, 130.9, 129.5, 128.0, 126.0, 124.1, 122.4, 121.2, 121.1, 115.6 ppm; HRMS (ESI-MS): *m/z* $[M+H]^+$ calcd for $C_{24}H_{16}NO_2$: 350.1181, found: 350.1172.

4.1.2.2. 4-(4-Hydroxyphenyl)-2-p-tolyl-5H-indeno[1,2-b]pyridin-5-one (H2), Scheme 1

Yield (8.7%): m.p: 273-276°C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ 9.95 (s, 1H, 4-phenyl 4-OH), 8.18 (d, 2H, Ar-H, *J* = 8.1 Hz), 7.91 (d, 1H, Ar-H, *J* = 7.3 Hz), 7.72 (s, 1H, indenopiridine H-3), 7.69-7.62 (m, 5H, Ar-H), 7.33 (d, 2H, Ar-H, *J* = 8.4 Hz), 6.86 (d, 2H, Ar-H, *J* = 8.4 Hz), 2.36 ppm (s, 3H, 2-phenyl-CH_3-); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 191.1, 166.4, 160.3, 159.8, 149.8, 142.8, 140.8, 135.9, 135.6, 135.5, 132.0, 131.9, 130.2, 127.9, 126.1, 124.1, 122.2, 121.1, 120.7, 115.5, 21.7 ppm; HRMS (ESI-MS): *m/z* $[M+H]^+$ calcd for $C_{25}H_{16}NO_2$: 362.1181, found: 362.1176.

4.1.2.3. 4-(4-Hydroxyphenyl)-2-(4-methoxyphenyl)-5H-indeno[1,2-b]pyridin-5-one (H3), Scheme 1

Yield (19.7%): m.p: 282-284°C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ 9.90 (s, 1H, 4-phenyl 4-OH), 8.27 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.92 (d, 1H, Ar-H, *J* = 7.3 Hz), 7.69 (s, 1H, indenopiridine H-3), 7.65-7.62 (m, 5H, Ar-H), 7.07 (d, 2H, Ar-H, *J* = 8.8 Hz), 6.86 (d, 2H, Ar-H, *J* = 8.8 Hz), 3.83 ppm (s, 3H, 2-phenyl-OCH_3); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 191.1, 166.5, 161.8, 160.2, 159.7, 149.8, 142.8, 135.8, 135.7, 132.0, 131.8, 130.6, 129.7, 126.2, 124.0, 121.7, 121.2,

120.0, 115.5, 114.9, 56.0 ppm; HRMS (ESI-MS): *m/z* $[M+H]^+$ calcd for $C_{25}H_{18}NO_3$: 380.1287, found: 380.1272.

4.1.2.4. 4-(4-Hydroxyphenyl)-2-(4-fluorophenyl)-5H-indeno[1,2-b]pyridin-5-one (H4), Scheme 1

Yield (28.8%): m.p: 314-317°C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ 9.97 (s, 1H, 4-phenyl 4-OH), 8.37-8.33 (m, 2H, Ar-H), 7.92 (d, 1H, Ar-H, *J* = 7.3 Hz), 7.76 (s, 1H, indenopiridine H-3), 7.73-7.50 (m, 5H, Ar-H), 7.35 (t, 2H, Ar-H, *J* = 8.8 Hz), 6.86 ppm (d, 2H, Ar-H, *J* = 8.8 Hz); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 191.0, 166.4, 159.8, 159.2, 149.9, 142.7, 135.9, 135.6, 132.1, 131.9, 130.5, 130.4, 125.9, 124.1, 122.3, 121.3, 121.0, 116.5, 116.3, 115.5 ppm; HRMS (ESI-MS): *m/z* $[M+H]^+$ calcd for $C_{24}H_{15}NO_2F$: 368.1087, found: 368.1074.

4.1.2.5. 4-(4-Hydroxyphenyl)-2-(4-bromophenyl)-5H-indeno[1,2-b]pyridin-5-one (H5), Scheme 1

Yield (9.7%): m.p: 303-305°C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ 9.97 (s, 1H, 4-phenyl 4-OH), 8.24 (d, 2H, Ar-H, *J* = 8.4 Hz), 7.92 (d, 1H, Ar-H, *J* = 7.3 Hz), 7.79 (s, 1H, indenopiridine H-3), 7.73-7.53 (m, 7H, Ar-H), 6.87 ppm (d, 2H, Ar-H, *J* = 8.4 Hz); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 190.9, 166.4, 159.8, 159.0, 149.9, 142.7, 137.4, 135.9, 135.6, 132.5, 132.2, 131.9, 131.6, 130.0, 125.9, 124.7, 124.2, 122.7, 121.3, 115.5 ppm; HRMS (ESI-MS): *m/z* $[M+H]^+$ calcd for $C_{24}H_{15}NO_2Br$: 428.0286, found: 428.0270.

4.1.2.6. 4-(4-Hydroxyphenyl)-2-(4-chlorophenyl)-5H-indeno[1,2-b]pyridin-5-one (H6), Scheme 1

Yield (20.1%): m.p: 308-310°C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ 9.97 (s, 1H, 4-phenyl 4-OH), 8.31 (d, 2H, Ar-H, *J* = 8.1 Hz), 7.92 (d, 1H, Ar-H, *J* = 7.3 Hz), 7.78 (s, 1H, indenopiridine H-3), 7.72-7.51 (m, 7H, Ar-H), 6.86 ppm (d, 2H, Ar-H, *J* = 8.4 Hz); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 190.9, 166.4, 159.9, 158.9, 149.9, 142.7, 137.0, 135.9, 135.8, 135.6, 132.2, 131.6,

131.9, 129.8, 129.5, 125.9, 124.2, 122.6, 121.3, 115.5 ppm; HRMS (ESI-MS): *m/z* $[M+H]^+$ calcd for $C_{24}H_{15}NO_2Cl$: 384.0791, found: 384.0773.

4.1.2.7. 4-(4-Hydroxyphenyl)-2-(3-hydroxyphenyl)-5H-indeno[1,2-b]pyridin-5-one (H7), Scheme 1

Yield (41.1%): m.p: 315-316°C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ 9.90 (s, 1H, 4-phenyl 4-OH), 9.64 (s, 1H, 2-phenyl 3-OH), 7.88 (d, 1H, Ar-H, *J* = 7.3 Hz), 7.17-7.50 (m, 8H, Ar-H, indenopiridine H-3), 7.31 (t, 1H, Ar-H, *J* = 7.7Hz), 6.90 (d, 2H, Ar-H, *J* = 8.8 Hz), 6.86 ppm (d, 1H, Ar-H, *J* = 8.4 Hz); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 191.1, 166.4, 160.5, 159.7, 158.4, 149.8, 142.7, 139.6, 135.9, 135.6, 133.1, 132.1, 131.8, 130.6, 126.1, 124.1, 122.4, 121.2, 118.9, 117.9, 115.6, 114.6 ppm; HRMS (ESI-MS): *m/z* $[M+H]^+$ calcd for $C_{24}H_{16}NO_3$: 366.1130, found: 366.1122.

4.1.2.8. 2,4-Bis(4-hydroxyphenyl)-5H-indeno[1,2-b]pyridin-5-one (H8), Scheme 1

Yield (16.4%): m.p: 336-338°C: ^{1}H NMR (400 MHz, [D_6]DMSO): δ 10.03 (s, 1H, 4-phenyl 4-OH), 9.93 (s, 1H, 2-phenyl 4-OH), 8.16 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.89 (d, 1H, Ar-H, *J* = 7.3 Hz), 7.70-7.49 (m, 6H, Ar-H, indenopiridine H-3), 6.91-6.85 ppm (m, 4H, Ar-H); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 191.1, 166.5, 160.5, 160.4, 159.7, 149.7, 142.8,135.9, 135.7, 131.9, 131.8, 129.8, 129.1, 126.3, 123.9, 121.4, 121.1, 119.6, 116.3, 115.5 ppm; HRMS (ESI-MS): *m/z* $[M+H]^+$ calcd for $C_{24}H_{16}NO_3$: 366.1130, found: 366.1122.

4.2. Biological activity

4.2.1. Cytotoxicity evaluation

The cytotoxicities of the compounds **H** and **H1-H8** were assayed towards human tumour cell lines [gingival carcinoma (Ca9–22), oral squamous cell carcinoma (HSC-2, HSC-3, HSC- 4)] and human normal oral cells [gingival fibroblasts (HGF), periodontal ligament fibroblasts (HPLF) and pulp cells (HPC)] as described [3, 5-8, 31,32]. In brief, all cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS). The following concentrations of the

compounds in dimethylsulfoxide (DMSO) were added to the medium and incubated at 37° C for 48 h: **H** and **H1**-**H8** (0.32, 1, 3.2, 10, 31.6, 100, 316 and 1000 mmol/L), melphalan (3.12, 6.25, 12.5, 25. 50, 100, 200 and 400 mmol/L) and 5-FU (7.8, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 mmol/L). The media that contained the same concentration of DMSO (0.0078, 0.156, 0.03125, 0.0625, 0.125, 0.25, 0.5 or 1%) were used as controls since DMSO above 0.25% is cytotoxic. The viable cell numbers were determined by the MTT method. The CC_{50} values were determined from dose-response curves.

4.2.2. Carbonic anhydrase enzyme assay

The Carbonic Anhydrase (CA) I and II isoenzymes were purified from fresh human blood erythrocytes using by Sepharose-4B-LTyrosine-sulfanilamide affinity chromatography [9, 13, 14, 33-39]. This method contains the purification of CA isoenzymes via a single step described previously. CA isoenzyme activity was determined spectrophotometricaly at 348 nm as described by Verpoorte et al. [40]. According to this method the absorbance changes were measured during the time of 3 min at 25°C as p-nitrophenylacetate (PNA) converted to 4-nitrophenylate ion. Bradford method was used to quantify the amount of protein during the purification steps [41]. Bovine serum albumin was used as standard protein [42]. After the purification process of the CA isoenzymes, SDSpolyacrylamide gel electrophoresis (SDS–PAGE) has been carried out. According to literature [9, 33-39] stacking and resolving gel containing 3% and 10% acrylamide, and 0.1% SDS was used for running the process using a Minigel system (Mini-PROTEAN system Casting stand, Catalog 1658050, Bio-Rad Laboratories, Inc. China). The method used for visualization of protein has been explained in detail in our previous studies [43]. According to this method, the gel was fixed then stained with Coomassie Brilliant Blues R-250 later on the gel stained by using standard methods for detecting protein bands that are belong to purified CA isoenzymes.

Declaration of Interest

The authors report no conflict of interest and are responsible for the contents and writing of the paper.

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Captions

Scheme 1. Synthesis of chalcone analogue **H** and azafluorenone derivatives **H1**-**H8**.

Table 1. Cytotoxicity of the compunds **H**, **H1**-**H8.**

Table 2. Human CA isoenzymes (hCA I and II) inhibition values by the compounds **H**-**H8**.

References

[1] D.B. Longley, P.G. Johnston, Molecular mechanisms of drug resistance, J. Pathol. 205(2) (2005) 275-92

[2] I. Gulcin, Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid), Toxicol. 217(2-3) (2006) 213-20

[3] C. Yamali, H.I .Gul, H. Sakagami, C.T. Supuran, Synthesis and bioactivities of halogen bearing phenolic chalcones and their corresponding bis Mannich bases, J. Enzyme. Inhib. Med. Chem. 31(sup4) (2016) 125-31

[4] C. Yamali, M. Tugrak, H.I. Gul, M. Tanc, C.T. Supuran, The inhibitory effects of phenolic Mannich bases on carbonic anhydrase I and II isoenzymes, J. Enzyme. Inhib. Med. Chem. 31(6) (2016) 1678-81

[5] M. Tugrak, C. Yamali, H. Sakagami, H.I. Gul, Synthesis of mono Mannich bases of 2-(4-hydroxybenzylidene)-2,3-dihydroinden-1-one and evaluation of their cytotoxicities, J. Enzyme. Inhib. Med. Chem. 31(5) (2016) 818-23

[6] M. Tugrak, H.I. Gul, H. Sakagami, Synthesis and cytotoxicities of 2-[4-hydroxy-(3,5-bis-aminomethyl)-benzylidene]-indan-1-ones, Lett. Drug. Des. Discov. 12(10) (2015) 806-12

[7] M. Tugrak, H.I. Gul, H. Sakagami, E. Mete, Synthesis and anticancer properties of mono Mannich bases containing vanillin moiety, Med. Chem. Res. 26(7) (2017) 1528-34

[8] S. Bilginer, H.I. Gul, E. Mete, U. Das, H. Sakagami, N. Umemura, et al., 1-(3-Aminomethyl-4-hydroxyphenyl)-3-pyridinyl-2-propen-1-ones: A novel group of tumour-selective cytotoxins, J. Enzyme. Inhib. Med. Chem. 28(5) (2013) 974-80

[9] H.I. Gul, M. Tugrak, H. Sakagami, P. Taslimi, I. Gulcin, C.T. Supuran, Synthesis and bioactivity studies on new 4-(3-(4-Substitutedphenyl)-3a,4-dihydro-3H-indeno[1,2-c]pyrazol-2-yl) benzenesulfonamides, J. Enzyme. Inhib. Med. Chem. 31(6) (2016) 1619-24

[10] K.O. Yerdelen, H.I. Gul, H. Sakagami, N. Umemura, M. Sukuroglu, Synthesis and cytotoxic activities of a curcumin analogue and its bis-Mannich derivatives, Lett. Drug. Des. Discov. 12(8) (2015) 643-9

[11] K.O. Yerdelen, H.I. Gul, H. Sakagami, N. Umemura, Synthesis and biological evaluation of 1,5-bis(4-hydroxy-3-methoxyphenyl)penta-1,4-dien-3-one and its aminomethyl derivatives, J. Enzyme. Inhib. Med. Chem. 30(3) (2015) 383-8

[12] I. Gulcin, S. Beydemir, Phenolic compounds as antioxidants: carbonic anhydrase isoenzymes inhibitors, Mini Rev. Med. Chem. 13(3) (2013) 408-30

[13] H.I. Gul, Z. Yazici, M. Tanc, C.T. Supuran, Inhibitory effects of benzimidazole containing new phenolic Mannich bases on human carbonic anhydrase isoforms hCA I and II. J. Enzyme. Inhib. Med. Chem. 31(6) (2016) 1540-4

[14] H.I. Gul, C. Yamali, A.T. Yasa, E. Unluer, H. Sakagami, M. Tanc, et al., Carbonic anhydrase inhibition and cytotoxicity studies of Mannich base derivatives of thymol, J. Enzyme. Inhib. Med. Chem. 31(6) (2016) 1375-80

[15] C.T. Supuran, Structure and function of carbonic anhydrases, The Biochem J. 473(14) (2016) 2023-32

[16] A. Scozzafava, P. Kalin, C.T. Supuran, I. Gulcin, S.H. Alwasel, The impact of hydroquinone on acetylcholine esterase and certain human carbonic anhydrase isoenzymes (hCA I, II, IX, and XII), J. Enzyme. Inhib. Med. Chem. 30(6) (2015) 941-6

[17] A. Innocenti, S. Beyza Ozturk Sarikaya, I. Gulcin, C.T. Supuran, Carbonic anhydrase inhibitors. Inhibition of mammalian isoforms I-XIV with a series of natural product polyphenols and phenolic acids, Bioorg. Med. Chem. 18(6) (2010) 2159-64

[18] S.K. Nair, P.A. Ludwig, D.W. Christianson, 2-Site binding of phenol in the active-site of human carbonic anhydrase structural implications for substrate association, J. Am. Chem. Soc. 116 (8) (1994) 3659-60

[19] C.T. Supuran, Carbonic anhydrases: novel therapeutic applications for inhibitors and activators, Nat. Rev. Drug. Discov, 2 (2008) 168-81

[20] G. De Simone, C.T. Supuran, (In)organic anions as carbonic anhydrase inhibitors. J. Inorg. Biochem. 111 (2012) 117-29

[21] S. Bilginer, E. Unluer, H.I. Gul, E. Mete, S. Isik, D. Vullo, et al., Carbonic anhydrase inhibitors. Phenols incorporating 2-or 3-pyridyl-ethenylcarbonyl and tertiary amine moieties strongly inhibit Saccharomyces cerevisiae beta-carbonic anhydrase, J. Enzyme. Inhib. Med. Chem. 29(4) (2014) 495-9

[22] A. Cave, M. Leboeuf, P.G. Waterman, S.W. Pelletier, In Alkaloids:Chemical and Biological Perspective. In: Ed.;Wiley, editor. London: Ed.;Wiley; (1987) p:245

[23] G.J. Arango, D. Cortes, B.K. Cassels, A. Cave, C. Merienne. Azafluorenones from Oxandra cf. major and biogenetic considerations, Phytochem. 26 (1987) 2093

[24] M.O.F. Goulart, A.E.G. Sant'ana, A.B. de Oliveira, G.G. de Oliveira, J.G.S. Maia, Azafluorenones and azaanthraquinone from Guatteria dielsiana, Phytochem. 25(7) (1986) 1691-1695

[25] H.T. Van, W.J. Cho, Structural modification of 3-arylisoquinolines to isoindolo[2,1-b]isoquinolinones for the development of novel topoisomerase 1 inhibitors with molecular docking study, Bioorg. Med. Chem. Lett.19(9) (2009) 2551-4

[26] X. Xiao, M, Cushman. An ab initio quantum mechanics calculation that correlates with ligand orientation and DNA cleavage site selectivity in camptothecin-DNA-topoisomerase I ternary cleavage complexes, J. Am. Chem. Soc. 127(28) (2005) 9960-1

[27] T.M. Kadayat, C. Park, K.Y. Jun, T.B. Thapa Magar, G. Bist, H.Y. Yoo, et al., Hydroxylated 2,4-diphenyl indenopyridine derivatives as a selective non-intercalative topoisomerase IIalpha catalytic inhibitor, Eur. J. Med. Chem. 90 (2015) 302-14

[28] T.M. Kadayat, C. Song, Y. Kwon, E.S. Lee, Modified 2,4-diaryl-5H-indeno[1,2-b]pyridines with hydroxyl and chlorine moiety: Synthesis, anticancer activity, and structure-activity relationship study, Bioorg. Chem. 62 (2015) 30-40

[29] T.M. Kadayat, C. Song, S. Shin, T.B. Magar, G. Bist, A. Shrestha, et al., Synthesis, topoisomerase I and II inhibitory activity, cytotoxicity, and structure-activity relationship study of 2-phenyl- or hydroxylated 2-phenyl-4-aryl-5H-indeno[1,2-b]pyridines, Bioorg. Med. Chem. 23(13) (2015) 3499-512

[30] S. Inayama, K. Mamoto, T. Shibata, T. Hirose, Structure and antitumor activity relationship of 2-arylidene-4-cyclopentene-1, 3-diones and 2-arylideneindan-1, 3-diones, J. Med. Chem. 19(3) (1976) 433-6

[31] C. Yamali, H.I. Gul, D.O. Ozgun, H. Sakagami, N. Umemura, C. Kazaz, et al., Synthesis and cytotoxic activities of difluoro-dimethoxy chalcones, Anti-cancer. Agents. Med. Chem. 17(10) (2017) 1426-33

[32] H.I. Gul, M. Tugrak, H. Sakagami, Synthesis of some acrylophenones with *N*-methylpiperazine and evaluation of their cytotoxicities, J. Enzyme. Inhib. Med. Chem. 31(1) (2016) 147-51

[33] I. Gulcin, A. Scozzafava, C.T. Supuran, H. Akincioglu, Z. Koksal, F. Turkan, et al., The effect of caffeic acid phenethyl ester (CAPE) on metabolic enzymes including acetylcholinesterase, butyrylcholinesterase, glutathione S-transferase, lactoperoxidase, and carbonic anhydrase isoenzymes I, II, IX, and XII, J. Enzyme. Inhib. Med. Chem. 31(6) (2016) 1095-101

[34] D.O. Ozgun, C. Yamali, H.I. Gul, P. Taslimi, I. Gulcin, T. Yanik, et al., Inhibitory effects of isatin Mannich bases on carbonic anhydrases, acetylcholinesterase, and butyrylcholinesterase, J. Enzyme. Inhib. Med. Chem. 31(6) (2016) 1498-501

[35] K. Kucukoglu, F. Oral, T. Aydin, C. Yamali, O. Algul, H. Sakagami, et al., Synthesis, cytotoxicity and carbonic anhydrase inhibitory activities of new pyrazolines, J. Enzyme. Inhib. Med. Chem. 31(sup4) (2016) 20-4

[36] E. Mete, B. Comez, H.I Gul, I. Gulcin, C.T. Supuran, Synthesis and carbonic anhydrase inhibitory activities of new thienyl-substituted pyrazoline benzenesulfonamides, J. Enzyme. Inhib. Med. Chem. 31(sup2) (2016) 1-5

[37] H.I. Gul, E. Mete, P. Taslimi, I. Gulcin, C.T. Supuran, Synthesis, carbonic anhydrase I and II inhibition studies of the 1,3,5-trisubstituted-pyrazolines, J. Enzyme. Inhib. Med. Chem. 32(1) (2017) 189-92

[38] H.I. Gul, A. Demirtas, G. Ucar, P. Taslimi, I. Gulcin, Synthesis of Mannich bases by two different methods and evaluation of their acetylcholine esterase and carbonic anhydrase inhibitory activities, Lett. Drug. Des. Discov. 14(5) (2017) 573-80

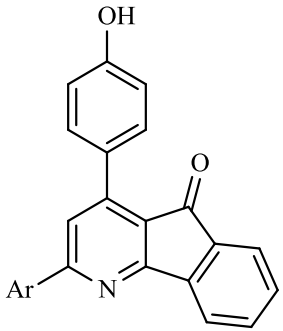
[39] C. Yamali, H.I. Gul, A. Ece, P. Taslimi, I. Gulcin, Synthesis, molecular modeling, and biological evaluation of 4-[5-aryl-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl] benzenesulfonamides toward acetylcholinesterase, carbonic anhydrase I and II enzymes, Chem. Biol. Drug. Des. 91(4) (2018) 854-866

[40] J.A. Verpoorte, S. Mehta, J.T. Edsall, Esterase activities of human carbonic anhydrases B and C, J. Biol. Chem. 242(18) (1967) 4221-9

[41] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248-54

[42] H. Lineweaver, D. Burk, The determination of enzyme dissoci-ation constants. J. Am. Chem. Soc. 56 (1934) 658-66

[43] H.I. Gul, C. Yamali, F. Yesilyurt, H. Sakagami, K. Kucukoglu, I. Gulcin, et al., Microwave-assisted synthesis and bioevaluation of new sulfonamides, J. Enzyme. Inhib. Med. Chem. 32(1) (2017) 369-74



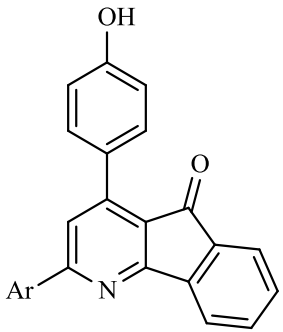
H1-H8

Ar: C_6H_5 (**H1),** 4-$CH_3C_6H_4$ (**H2),** 4-$CH_3OC_6H_4$ (**H3),** 4-FC_6H_4 (**H4),** 4-BrC_6H_4 (**H5),** 4-ClC_6H_4 (**H6),** 3-HOC_6H_4 (**H7),** 4-HOC_6H_4 (**H8**)

Table 1. Cytotoxicity of the compunds **H**, and **H1-H8**

	Tumour cell lines CC_{50} (µM)										Non-tumour cell lines CC_{50} (µM)				PSEx100
Compound	**Ca9-22 (A)**	**SI**	**HSC-2 (B)**	**SI**	**HSC-3 (C)**	**SI**	**HSC-4 (D)**	**SI**	**E (mean)**	**J**	**HGF (F)**	**HPLF (G)**	**HPC (H)**	**I (mean)**	**J/E**
H	269	2.1	182	3.2	219	2.6	182	3.2	213±41	2.8	968	448	320	579±343	1.3
H1	8.5	0.6	2.8	1.9	9.9	0.6	3	1.8	6.1±3.7	1.2	6.6	7.1	2.8	5.5±2.4	19.7
H2	19	1.1	11	1.8	18	1.1	19	1.1	17±3.9	1.3	21	20	18	20±1.5	7.6
H3	24	1.0	11	2.3	21	1.2	15	1.7	18±5.9	1.6	25	28	22	25±3.0	9.0
H4	46	0.1	17	0.4	5.9	1.1	21	0.3	22±17	0.5	5.7	7.1	6.3	6.4±0.7	2.3
H5	13	1.5	5.2	3.6	9.1	2.1	7.9	2.4	8.8±3.2	2.4	13	21	23	19±5.3	27.3
H6	13	1.8	8.1	2.8	7.7	2.9	8.4	2.7	9.3±2.5	2.6	22	38	8.5	23±15	28.0
H7	19	1.3	7.5	3.2	8	3.0	7.9	3.0	11±5.6	2.6	9.7	32	30	24±12	23.6
H8	24	1.5	9.1	4.0	12	3.0	9.6	3.8	14±7.0	3.1	25	52	32	36±14	22.1
Average	43.39	1.22	28.19	2.58	34.51	1.96	30.42	2.22	35.47	2.01	121.78	72.58	51.4	81.99	15.7
Melphalan	22.6	6.5	8.5	17.3	5.6	26.2	11.9	12.3	12.2±7.4	15.6	140.0	179.0	123.0	147.0±29.0	127.7
5-FU*	29	>34.5	13	>77.0	16	>62.5	13	>77.0	18±7.6	62.8	>1000	>1000	>1000	>1000	348.8

CC_{50} values refer to the concentrations of the compounds in micromoles which reduce the viable cell number by 50%. Selectivity index (SI) figures were generated which are quotients of the average CC_{50} values of non-malignant cells and CC_{50} figure of a compound towards a specific cell line. A potency selectivity expression (PSE) was devised which is reciprical of the average CC_{50} value (a measure of potency) and the average SI figure (a determination of tumour selectivity) (Column J/ Column E x 100).
*5-FU (5-Fluorouracil) was also used as a reference drug. µM : micromolar



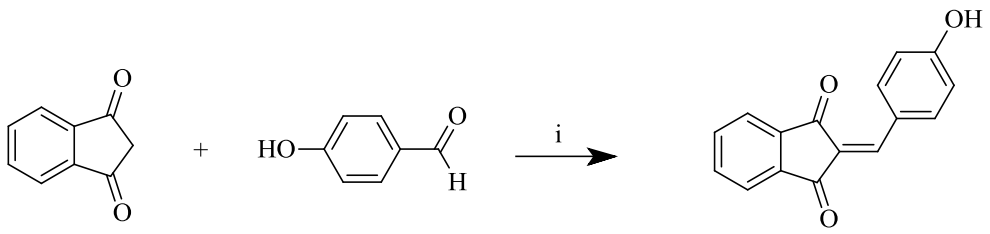
H1-H8

Ar: C_6H_5 (**H1),** 4-$CH_3C_6H_4$ (**H2),** 4-$CH_3OC_6H_4$ (**H3),** 4-FC_6H_4 (**H4),** 4-BrC_6H_4 (**H5),** 4-ClC_6H_4 (**H6),** 3-HOC_6H_4 (**H7),** 4-HOC_6H_4 (**H8)**

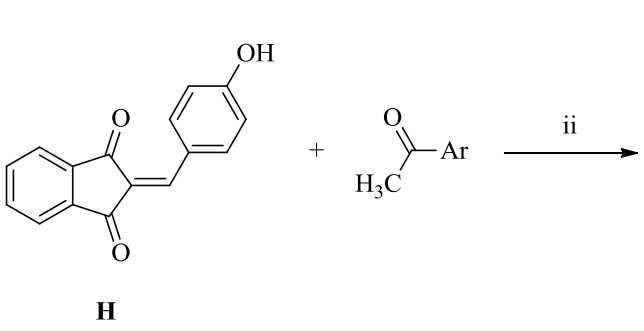
Table 2. Human CA isoenzymes (hCA I and II) inhibition values by the compounds **H-H8**

	IC_{50} (nM)				K_I (nM)	
Compound	**hCA I**	**r^2**	**hCA II**	**r^2**	**hCA I**	**hCA II**
H	87.721	0.9738	10.661	0.9277	4.7987±1.282	10.533±3.197
H1	61.875	0.9688	67.281	0.9450	6.8316±2.022	3.5280±1.135
H2	70.020	0.9292	71.443	0.9792	4.6577±1.022	6.7563±1.535
H3	58.728	0.9495	74.516	0.9455	7.2645±3.140	5.6070±1.671
H4	68.613	0.9646	73.723	0.9626	8.4757±2.554	7.9153±2.016
H5	59.230	0.9689	59.741	0.9705	5.1777±1.463	3.8242±0.874
H6	61.327	0.9791	67.281	0.9725	5.0125±1.258	7.2065±1.740
H7	54.140	0.9103	68.613	0.9832	7.5103±3.442	3.5061±0.911
H8	73.723	0.9399	76.153	0.9880	6.6933±1.386	6.1730±1.155
AZA	197.304	0.9889	115.50	0.9719	183.390±19.71	104.60±27.60

AZA: Acetazolamide, reference compound used.
H: Starting compound for the synthesis of **H1**-**H8**, **H1**-**H8**: New azaflorenones synthesized



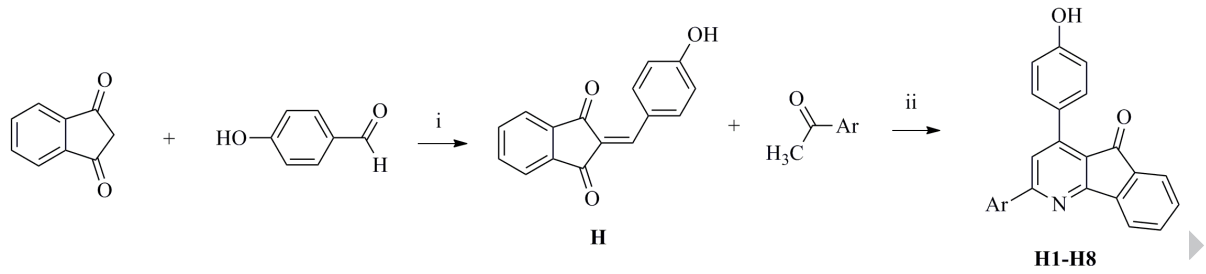
H



H **H1-H8**

Ar: C_6H_5 (**H1),** 4-$CH_3C_6H_4$ (**H2),** 4-$CH_3OC_6H_4$ (**H3),** 4-FC_6H_4 (**H4),** 4-BrC_6H_4 (**H5),** 4-ClC_6H_4 (**H6),** 3-HOC_6H_4 (**H7),** 4-HOC_6H_4 (**H8)**
Reagent and conditions: i) EtOH, aqueous solution of NaOH 10%, ii) CH_3COONH_4, 120°C, 200 Watt, DMF

Scheme 1. Synthesis of chalcone analogue **H** and azafluorenone derivatives **H1**-**H8**.
H: Starting compound for the synthesis of the **H1**-**H8**



Ar: C_6H_5 (**H1**), 4-$CH_3C_6H_4$ (**H2**), 4-$CH_3OC_6H_4$ (**H3**), 4-FC_6H_4 (**H4**), 4-BrC_6H_4 (**H5**), 4-ClC_6H_4 (**H6**), 3-HOC_6H_4 (**H7**), 4-HOC_6H_4 (**H8**)
Reagent and conditions: i) EtOH, aqueous solution of NaOH 10%, ii) CH_3COONH_4, 120°C, 200 Watt, DMF

Scheme 1. Synthesis of chalcone analogue **H** and azafluorenone derivatives **H1**-**H8**.
H: Starting compound for the synthesis of the **H1**-**H8**