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Design and synthesis of indole integrated aurones as potent anti breast cancer agents

KEYWORDS

Aurone , Anticancer, Flavnoides , Indole, MCF-7

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ABSTRACT *A divers library of indole integrated aurones(5a-n) were synthesized by knoevenagel condensation of 4,6 -dimethoxybenzofuran-3(2H)-one (3) with various indole aldehydes (4a-n) in the presence of sodium hydroxide. Structural investigation of the synthesized compounds was carried out by IR, 1H NMR and mass spectral data. All the synthesized compounds were in vitro screened for their anticancer potential against breast cancer cell lines MCF-7 and MDA-MB-231 using adriamycin as a reference standard. Preliminary in vitro studies revealed that compound 5c possess excellent activity against MCF-7 (GI50 < 10μM) as good as standard drug adriamycin (GI50 < 10μM).*

INTRODUCTION

Naturally occurring flavonoids have shown enormous biological potential in recent years, in particular the subclass of flavonoids known as aurones. Previous studies have shown that aurones display anti-cancer activity as well as a variety of other pharmacological activities, including anti-inflammatory, antioxidants, antifungal and anti-viral properties [1-5]. More specifically, these studies indicate that aurones show potential in inhibiting cyclooxygenase-2 activity (COX-2), which plays an integral part of inflammation and its allied diseases, such as cancer. Aurones can be used as potential cancer chemotherapy agents and as inhibitors of an enzyme involved in the metabolism of thyroid hormones [6]. They have also been reported to be antiproliferative agents interfering with G2/M phase of the cell cycle [7], tyrosinaseinhibitors [8] and as potentially useful imaging agents for detecting -amyloid plaques in Alzheimer's disease [9]. Aurone derivatives from plant extracts have been used in the treatment of thyroid diseases [10]. The aurones isolated from Uvariahamiltonii displayed anticancer activity [11].

An indole is an aromatic heterocyclic compound which has its heterobicyclic configuration as a six-membered ring fused to a five-membered pyrrole ring. They are a very important category of compounds that play a vital role in cell physiology and are probable intermediates for numerous biological reactions. Indole derivatives possess diverse therapeutic properties such as anticancer [12], antioxidant [13], antirheumatoidal [14], anti-HIV [15, 16], antimicrobial [17-19], anti-inflamatory [20], analgesic [21], antipyretic [22], anticonvulsant [23,24], and cardiovascular [25], activities.

Based on these attractive biological activity profiles of aurone and indole scaffolds, we made an attempt to synthesize a diverse library of indole based aurones as potent anticaner agents.

Experimental section

General procedure for the synthesis of indole bases aurones (5a-n)

4,6-dimethoxybenzofuran-3(2H)-one 3 (1 eq) was dissolved in ethanol (10 ml) under stirring. To this was added NaOH (3 eq, with a minimum of water) and stirred for 5 minute. To this reaction mixture was then added indole aldehyde 4 (1 eq) and stirring continued at room temperature for 48 h. Reaction was monitored by TLC. After completion of reaction, the reaction mixture was poured over crushed ice and acidified with acetic acid. The separated product was filtered and washed with cold water. Crude product was oven dried and recrystallized from alcohol to obtain the pure product 5.

Spectral data of representative compounds:

(Z)-2-((1H-pyrrolo[2,3-b]pyridin-3-yl)methylene)-4,6-dimethoxybenzofuran-3(2H)-one (5b): IR (cm-1): 3167, 1683, 1637, 1608, 1590; 1H NMR (200 MHz, CDCl3): = 3.95 (s, 3H, OCH3), 4.0 (s, 3H, OCH3), 6.19 (d, 1H, J = 2Hz, ArH), 6.48 (d, 1H, J = 2Hz, ArH), 7.14 (s, 1H, ArH), 7.28-7.35 (m, 1H, ArH), 8.01 (s, 1H, =CH), 8.31 (d, 1H, J = 8Hz, ArH), 8.43 (d, 1H, J = 6Hz, ArH), 8.68 (s, 1H, NH); MS (ESI): m/z = 323 (M+1).

(Z)-4,6-dimethoxy-2-((2-methyl-1H-indol-3-yl)methylene)benzofuran-3(2H)-one (5c): IR (cm-1): 3151, 1685, 1651, 1608, 1585; 1H NMR (200 MHz, CDCl3): = 2.67 (s, 3H, ArCH3), 3.97 (s, 3H, OCH3), 3.99 (s, 3H, OCH3), 6.18 (d, 1H, J = 2Hz, ArH), 6.46 (d, 1H, J = 2Hz, ArH), 7.13 (s, 1H, NH), 7.25-7.36 (m, 3H, ArH), 8.42 (m, 1H, ArH), 8.56 (m, 1H, ArH); MS (ESI): m/z = 336 (M+1).

(Z)-2-((1-(4-chlorobenzyl)-1H-indol-3-yl)methylene)-4,6-dimethoxybenzofuran-3(2H)-one (5e): IR (cm-1): 3080, 1675, 1632, 1609; 1H NMR (200 MHz, CDCl3): = 3.95 (s, 3H, OCH3), 4.0 (s, 3H, OCH3), 5.42 (s, 2H, CH2), 6.17 (d, 1H, J = 2Hz, ArH), 6.41 (d, 1H, J = 2Hz, ArH), 7.12 (d, 2H, J = 8Hz, ArH), 7.30-7.36 (m, 6H, ArH), 7.96-8.02 (m, 2H, ArH, =CH); MS (ESI): m/z = 446 (M+1).

(Z)-2-((1-(2,4-dichlorobenzyl)-1H-indol-3-yl)methylene)-4,6-dimethoxybenzofuran-3(2H)-one (5f): IR (cm-1): 3091, 2939, 2836, 1671, 1644, 1600, 1589; 1H NMR (200 MHz, CDCl3): = 3.95 (s, 3H, OCH3), 4.01 (s, 3H, OCH3), 5.51 (s, 2H, CH2), 6.18 (d, 1H, J = 2Hz, ArH), 6.41 (d, 1H, J = 2Hz, ArH), 7.14 (d, 2H, J = 8Hz, ArH), 7.28-7.35 (m, 5H, ArH), 7.99-8.04 (m, 2H, ArH, =CH); MS (ESI): m/z = 480 (M+1).

(Z)-2-((1-(2,4-dichlorobenzyl)-5-methoxy-1H-indol-3-yl)methylene)-4,6-dimethoxybenzofuran-3(2H)-one (5i): IR

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(cm-1): 3089, 2938, 1672, 1641, 1601, 1589; 1H NMR (200 MHz, CDCl3): = 3.89 (s, 3H, OCH3), 3.92 (s, 3H, OCH3), 3.96 (s, 3H, OCH3), 5.39 (s, 2H, CH2), 6.12 (d, 1H, J = 2Hz, ArH), 6.34 (d, 1H, J = 2Hz, ArH), 6.52 (d, 1H, J = 2Hz, ArH), 6.88 (d, 1H, J = 8Hz, ArH), 7.20 (d, 1H, J = 2Hz, ArH), 7.37 (d, 1H, J = 2Hz, ArH), 7.45 (d, 2H, J = 2Hz, ArH), 7.94 (s, 1H, =CH); MS (ESI): m/z = 510 (M+1).

(Z)-2-((1-(4-chlorobenzyl)-1H-pyrrolo[2,3-b]pyridin-3-yl)methylene)-4,6-dimethoxybenzofuran-3(2H)-one (5j): IR (cm-1): 2929, 2840, 1681, 1651, 1606, 1590 ; 1H NMR (200 MHz, CDCl3): = 13.85 (s, 3H, OCH3), 3.89 (s, 3H, OCH3), 5.48 (s, 2H, CH2), 6.06 (d, 1H, J = 2Hz, ArH), 6.30 (d, 1H, J = 2Hz, ArH), 7.02 (s, 1H, ArH), 7.10-7.25 (m, 5H, ArH), 7.89 (s, 1H, =CH), 8.20 (d, 1H, J = 8Hz, ArH), 8.33 (d, 1H, J = 6Hz, ArH); MS (ESI): m/z = 447 (M+1).

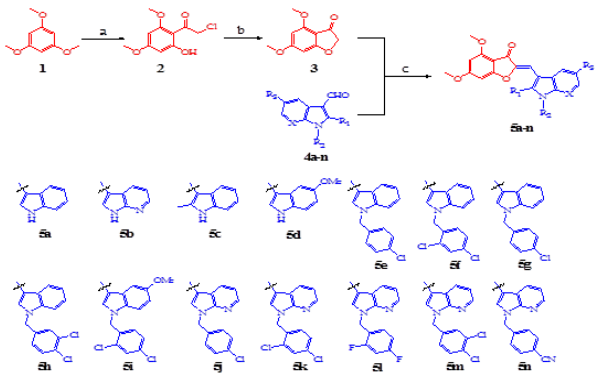
(Z)-2-((1-(2,4-dichlorobenzyl)-1H-pyrrolo[2,3-b]pyridin-3-yl)methylene)-4,6-dimethoxybenzofuran-3(2H)-one (5k): IR (cm-1): 3090, 3006, 2944, 1682, 1651, 1606, 1525 ; 1H NMR (200 MHz, CDCl3): = 3.96 (s, 3H, OCH3), 4.00 (s, 3H, OCH3), 5.69 (s, 2H, CH2), 6.17 (d, 1H, J = 2Hz, ArH), 6.40 (d, 1H, J = 2Hz, ArH), 6.87 (d, 1H, J = 8Hz, ArH), 7.14 (s, 1H, ArH), 7.28-7.32 (m, 1H, ArH), 7.49 (d, 1H, J = 2Hz, ArH), 8.07 (s, 1H, =CH), 8.33 (d, 1H, J = 8Hz, ArH), 8.44 (d, 1H, J = 6Hz, ArH); MS (ESI): m/z = 481 (M+1).

(Z)-2-((1-(2,4-difluorobenzyl)-1H-pyrrolo[2,3-b]pyridin-3-yl)methylene)-4,6-dimethoxybenzofuran-3(2H)-one (5l): IR (cm-1): 3027, 2979, 2842, 1676, 1651, 1602; 1H NMR (200 MHz, CDCl3): = 3.97 (s, 3H, OCH3), 4.00 (s, 3H, OCH3), 5.63 (s, 2H, CH2), 6.17 (d, 1H, J = 2Hz, ArH), 6.43 (d, 1H, J = 2Hz, ArH), 6.80-6.90 (m, 2H, ArH), 7.13 (s, 1H, ArH), 7.22-7.30 (m, 2H, ArH), 8.06 (s, 1H, =CH), 8.32 (d, 1H, J = 8Hz, ArH), 8.46 (d, 1H, J = 6Hz, ArH); MS (ESI): m/z = 449 (M+1).

(Z)-2-((1-(3,4-dichlorobenzyl)-1H-pyrrolo[2,3-b]pyridin-3-yl)methylene)-4,6-dimethoxybenzofuran-3(2H)-one (5m): IR (cm-1): 3086, 1687, 1641, 1613, 1589; 1H NMR (200 MHz, CDCl3): = 3.97 (s, 3H, OCH3), 4.01 (s, 3H, OCH3), 5.58 (s, 2H, CH2), 6.18 (d, 1H, J = 2Hz, ArH), 6.43 (d, 1H, J = 2Hz, ArH), 7.14 (s, 1H, ArH), 7.28-7.32 (m, 2H, ArH), 7.41-7.45 (m, 2H, ArH), 8.0 (s, 1H, =CH), 8.31 (d, 1H, J = 8Hz, ArH), 8.45 (d, 1H, J = 6Hz, ArH); MS (ESI): m/z = 481 (M+1).

(Z)-4-((3-((4,6-dimethoxy-3-oxobenzofuran-2(3H)-ylidene)methyl)-1H-pyrrolo[2,3-b]pyridin-1-yl)methyl)benzonitrile (5n): IR (cm-1): 3091, 2945, 2224, 1683, 1651, 1608, 1504; 1H NMR (200 MHz, CDCl3): = 3.95 (s, 3H, OCH3), 4.0 (s, 3H, OCH3), 5.69 (s, 2H, CH2), 6.16 (d, 1H, J = 2Hz, ArH), 6.39 (d, 1H, J = 2Hz, ArH), 7.13 (s, 1H, ArH), 7.30-7.38 (m, 2H, ArH), 7.66 (d, 2H, J = 8Hz, ArH), 8.01 (s, 1H, =CH), 8.30 (d, 1H, J = 8Hz, ArH), 8.43 (d, 1H, J = 6Hz, ArH); MS (ESI): m/z = 438 (M+1).

Synthesis of title compounds 5a-n was carried out by the Knoevenagel condensation of 4,6-dimethoxybenzofuran-3(2H)-one (3) with variety of indole aldehydes (4a-n) in the presence of sodium hydroxide in ethanol (Scheme 1). Compound 4,6-dimethoxybenzofuran-3(2H)-one (3) was prepared by Friedel-Craft acylation of 1,3,5-trimethoxy benzene with chloroacetyl chloride followed by the cyclization in the presence of potassium acetate as per the literature precedent [26,27]. Indole aldehydes (4a-n) were prepared by the Vilsmeier-Haack formylation of indole and azaindole using DMF-POCl3 followed by the alkylation using various benzyl halides in the presence of sodium hydride in dry DMF. Structural elucidation of all the newly synthesized compounds was carried out with the help of IR, 1H NMR and Mass Spectral data.



Scheme 1: Synthesis of indole integrated aurones; Reagents and conditions: (a) $ClCOCH_2Cl$, anh. $AlCl_3$, 0°C – rt, 48h; (b) CH_3COOK, EtOH, Reflux, 8h; (c) NaOH, EtOH, rt

Anticancer activity

All the synthesized indole based aurones (5a-n) were evaluated for their in vitro anticancer potential in human breast cancer cell lines MCF-7 and MDA-MB-231 by using the sulforhodamine B (SRB) assay method [28]. The most effective anticancer agent, adriamycin is used as a reference drug. Three parameters GI50, TGI and LC50 were determined during the screening process and the results are summarized in Table 1.

The GI50 values refer to the drug concentration that produces a 50% reduction of cellular growth compared with the untreated control cells. The TGI and LC50 values refer to the drug concentration required for total growth inhibition and killing 50% of the cells, respectively. GI50 values used to classify a compound's activity as follows: inactive, >100 μM; moderate, between >10 and <100 μM; and active, <10 μM.

Among the tested compounds most of the compounds were not significantly cytotoxic against MDA-MB-231, whereas significantly cytotoxic against MCF-7 compared to the standard drug tested, i.e. adriamycin, with the concentration of the drug that produced 50% inhibition of cell growth (GI50). Among all the compounds screened, compound 5c exhibited potent activity (GI50 = <10 μM) against the MCF-7 cell line which was almost as good as that of adriamycin (GI50 = <10 μM). Whereas, all other compounds were not possesses significant activity against MDA-MB-231 and MCF-7 cell lines. Compound 5c also possess significant total growth inhibitory (TGI) activity (TGI = <10 μM) as good as standard drug adriamycin (TGI = <10 μM).

Structure activity relationship (SAR) study revealed that the presence of methyl group at 2-position of indole is essential for activity. Compound 5c having methyl substituent at 2-position of the indole ring exhibited potent activity (GI50 = <10 μM, TGI = <10 μM) against MCF-7 cell line. The introduction of benzyl substituents on indole nitrogen not significantly enhances the activity.

Table 1. In vitro anticancer screening of indole integrated aurones (5a-n) against human breast cancer cell lines.a

Entry	MDA-MB-231	MCF-7				
	Lc50	TGI	GI_{50}	LC50[b]	TGI[c]	GI50[d]
5a	>80	>80	>80	>80	>80	>80
5b	>80	>80	>80	>80	>80	>80
5c	>80	>80	>80	>80	<10	<10
5d	>80	>80	>80	>80	>80	>80
5e	>80	>80	>80	>80	>80	>80
5f	>80	>80	>80	>80	>80	>80

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5g	>80	>80	>80	>80	>80	>80
5h	>80	>80	>80	>80	>80	>80
5i	>80	>80	>80	>80	>80	>80
5j	>80	>80	>80	>80	>80	>80
5k	>80	>80	>80	>80	>80	>80
5l	>80	>80	>80	>80	>80	>80
5m	>80	>80	>80	>80	>80	>80
5n	>80	>80	>80	>80	>80	>80
ADR	NE	<10	<10	20	<10	<10

a Concentrations in µg/ml; b Concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) calculated from [(Ti - Tz)/Tz]x100 = -50; c Drug concentration resulting in total growth inhibition (TGI) will calculated from Ti = Tz; d Growth inhibition of 50% (GI50) calculated from [(Ti - Tz)/(C - Tz)] x 100 = 50; NE = Not Estimated

CONCLUSIONS

In conclusion, we synthesized variety of indole based aurones and the structures were well elucidated by IR, 1H NMR, and mass spectral data. The newly synthesized compounds were in vitro screened for their anticancer activities against breast cancer cell lines (MCF-7 and MDA-MB-231). Among the compounds screened compounds 5c demonstrated potent anticancer potential against MCF-7, whereas all other compounds were not found to be significantly cytotoxic against both the cell lines. SAR study reveals that methyl-substitution at 2-position of indole show positive influence on anticancer potential.

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