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Original article

Synthesis and evaluation of novel marine bromopyrrole alkaloid-based hybrids as anticancer agents

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ABSTRACT

A series of twenty three novel hybrids of marine bromopyrrole alkaloids with chalcone, isoxazole and flavone structural features were synthesized and evaluated for in vitro anticancer activity by MTT assay against five human cancer cell lines. Among the synthesized chalcones, hybrids **4a** and **4h** (IC50 range: 0.18 μM–12.00 μM) showed anticancer activity against all the tested cancer cell lines. Promising cytotoxic activities were exhibited by flavones derivatives, **5a** and **5b** (0.41 μM–1.28 μM) against cell lines **PA1** and **KB403**. Isoxazole hybrids, **6b**–**6e** selectively inhibited oral and mouth cancer cell line **KB403**, among which **6c** (IC50 = 2.45 μM) was found to be most active.

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1. Introduction

The problem of cancer is increasing across the world making it a leading cause of deaths in economically developed and developing countries [1]. A great deal of efforts has been ongoing to treat various forms of cancer for decades; and until recently, chemoprevention is getting its due share of attention. Anticancer drugs show their cytotoxic activity through apoptosis [2]. The success of many existing anticancer drugs is restricted by their toxicity to normal cells. Among the small molecule drugs developed to treat cancer, chalcones represents promising group of compounds. Chalcones constitute an important class of natural products which serve as precursors for the preparation of various flavonoids and isoflavonoids. They contain open-chain flavones system in which two aromatic rings are joined by a three carbon α,β-unsaturated carbonyl system [3,4]. Natural and synthetic chalcones have displayed broad spectrum of biological activities such as antiinflammatory [5], antimicrobial [6], antifungal [7], antimalarial [8], antileishmanicidal [9], and anticancer agents [10–14]. Moreover, synthetic derivatives of chalcones, namely isoxazoles and flavones are well known for their anticancer activity [15–18].

Our group is working on design and synthesis of novel bioactive molecules using molecular hybridization approach [19]. In this light, we have reported synthesis and antimicrobial activities of hybrids of bromopyrrole alkaloids with 1,3,4-oxadiazole and 4-thiazolidinone moieties [20,21]. Bromopyrrole alkaloid is an important family of marine alkaloids having broad range of biological activities like anti-histaminic [22], antiserotonergic, antimicrobial, antitubercular [23], and antibiofilm agent [24]. Importantly these natural products also possess antineoplastic activity [25–27]. Most of these compounds are characterized by the presence of 4,5-dibromopyrrole ring with oroidin as their prototype alkaloid. From these observations and literature reports, novel hybrids of chalcone, isoxazole and flavone incorporating 4,5-dibromopyrrole as structural feature were designed. Further, the effect of *N*-methylation of pyrrole core on anticancer activity in the proposed hybrid was also studied. The synthesized hybrids were evaluated for their anticancer activity against five human cancer cell lines **MCF7** (hormone dependant breast cancer cells), **PA1** (ovary cancer cells), **WRL68** (liver cancer cells), **CaCO2** (colon cancer cells) and **KB403** (oral and mouth cancer cells) using MTT assay technique (Fig. 1).

2. Result and discussion

2.1. Chemistry

The target compounds were synthesized as illustrated in Scheme 1. 1-*H*-pyrrole and 1-methyl-pyrrole (**1a** and **1b**) were formylated using phosphorus oxychloride and anhydrous DMF in ethylene

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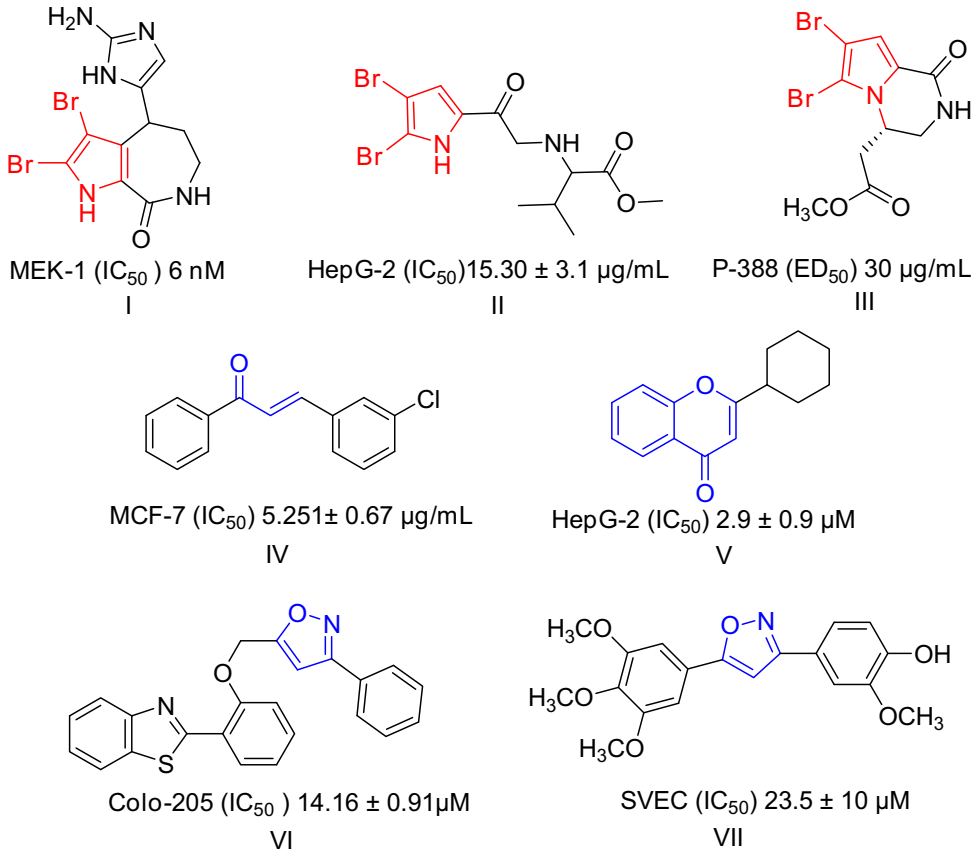


MEK-1 : Mitogen-activated protein kinase kinase 1
HepG-2 : Human liver carcinoma cell line
P-388 : Lymphocytic leukemia cells
MCF-7 : Breast cancer cell line
Colo-205: Human colon adenocarcinoma cell line
SVEC : Simian virus 40-transformed mouse endothelial cell line

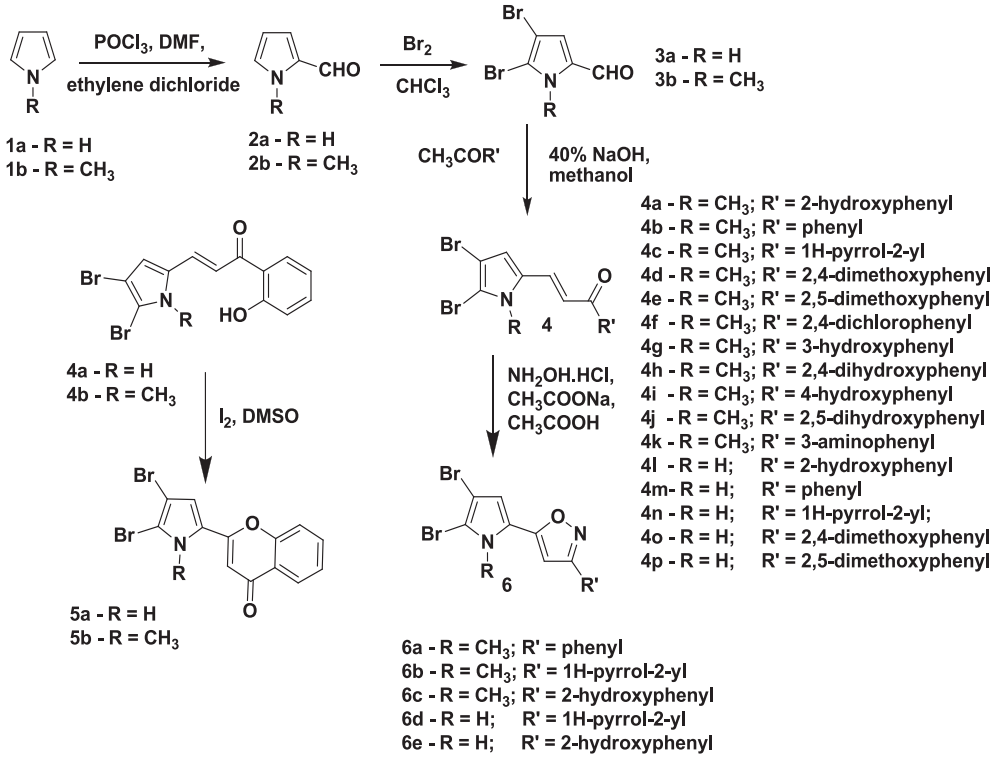
Fig. 1. Reported bromopyrrole alkaloids and chalcone, isoxazoles and flavone with anticancer activities.

dichloride to give 1*H*-pyrrole-2-carboxaldehyde **2a** and 1-methyl-pyrrole-2-carboxaldehyde **2b** [28]. The compounds **2a** and **2b** were brominated using *N*-bromosuccinimide in DMF to give corresponding dibromo derivatives, **3a** and **3b** [29]. Dibromopyrrole-2-carboxaldehydes (**3a** and **3b**) were converted into their respective chalcone derivatives **4** using aryl/heteroaryl ketones in methanolic NaOH (40%). Oxidation of compound **4a** and **4l** with iodine and DMSO furnishes respective flavones **5a** and **5b** [30]. Compounds **4b**, **4c**, **4i**, **4l** and **4n** were converted into their isoxazole derivatives **6a**–**6e** respectively by refluxing with anhydrous sodium acetate, acetic acid and hydroxylamine hydrochloride for 8 h [31].

Characterization of all the synthesized compounds was done by IR, ^{1}H NMR and MS and the results were in agreement with the proposed structures. (M+) molecular ion peak confirmed the molecular weight of the compounds while (M + 2) and (M + 4) peaks confirmed the presence of bromines in the structure. The ^{1}H NMR spectra of all compounds showed a single at δ 4.0–3.8 corresponding to N–CH_3 attached to pyrrole while N–H proton showed a peak at δ 12.6–12.2. Proton at third position of pyrrole ring resonated at δ 6.5–6.3. Protons at α and β unsaturated system of chalcones resonated at δ 7.6–7.8. In flavone derivatives, protons of aryl ring of chroman resonated at δ 7.5–8.0, while proton at third position of chroman ring resonated at δ 7.0. The proton at fourth position of isoxazole ring resonated between δ 7.0–7.1. The protons of substituted aryl ring were generally resonated between δ 7.7–6.4.

2.2. Biological activity

Anticancer activity data of synthesized compounds against five human cancer cell lines **MCF7** (hormone dependant breast cancer cells), **PA1** (ovary cancer cells), **WRL68** (liver cancer cells), **CaCO2** (colon cancer cells) and **KB403** (oral and mouth cancer cells) using MTT assay is given in Table 1. Among the twenty three tested hybrids, thirteen showed promising anticancer activity ($IC_{50} \leq 1.00$ µM) against at least one of the tested cell lines. From Table 1, some preliminary structure–activity relationships concerning the effect of substituents **R** and **R′** could be drawn. The anticancer profiles of the screened chalcones **4a**–**p** indicated that substitution on **R′** ring has significant effect on activity. For chalcones, **4a** ($IC_{50} = 0.33$ µM), **4c** ($IC_{50} = 2.11$ µM), **4d** ($IC_{50} = 0.64$ µM) and **4e** ($IC_{50} = 3.27$ µM), *N*-methylation of pyrrole core was found to be positive for anticancer activity against liver cancer cell line **WRL68** compared to corresponding non methylated analogues **4l**–**4p**. However *N*-methylation of pyrrole core in chalcones **4a**–**e** leads to decrease in anticancer activities against hormone dependent breast cancer cell line **MAF7** and ovary cancer cells **PA1** compared to corresponding non methylated analogues, except for hybrid **4c** containing pyrrole ring at **R′** position. All Chalcone hybrids **4a**–**e** showed good anticancer profile against hormone dependent breast cancer cells **MCF7** (IC_{50} range = 0.03–35.36 µM) where highest activity was shown by hybrid **4o** (**MCF7**, $IC_{50} = 0.03$ µM) indicating presence of 2,4-dimethoxy substitution and absence of *N*-methylation on pyrrole core leads to



Scheme 1. Synthesis of 4,5-dibromo-1*H*-pyrrole based chalcones and their derivatives.

increased cytotoxicity compared to other substituted chalcones. Similar cytotoxic activities were shown by chalcone hybrids against ovary cancer cells **PA1** (IC_{50} range = 0.32–2.85 μM) where highest activity was shown by **4o** (**PA1**, IC_{50} = 0.32 μM). Hybrid **4b**, **4g**, **4k** and **4m** were inactive at highest tested concentration against ovary cancer cells **PA1**. Chalcone hybrids **4a**, **4h** and **4i** showed good cytotoxicity against colon cancer cells **CaCO2** where hybrid **4a** showed highest activity (IC_{50} = 3.16 μM). All other chalcones were inactive against colon cancer cells **CaCO2**. In case of oral and mouth cancer cells **KB403**, chalcone hybrids **4a**, **4c** and **4h** showed good cytotoxity where highest potency was showed by **4c** (IC_{50} = 0.60 μM). Anticancer profile (IC_{50} range = 0.18–12.00 μM) shown by hybrids **4a** and **4h** against all the tested cancer cell lines indicated that presence of hydroxyl substituent at 2-postion of R′ ring is positive for the cytotoxicity but negative for selectivity.

Table 1
Cytotoxicity of chalcone and their derivatives against various human cancer cell lines by MTT assay.

Compound	Cancer cell lines				
	MCF7 IC_{50} (μM)	**PAI** IC_{50} (μM)	**WRL68** IC_{50} (μM)	**CaCO2** IC_{50} (μM)	**KB403** IC_{50} (μM)
4a	2.51	1.25	0.33	3.16	8.32
4b	35.36	–	–	–	–
4c	0.45	0.73	2.11	–	0.60
4d	0.08	0.52	0.64	–	–
4e	0.65	0.80	3.27	–	–
4f	1.84	1.00	14.67	–	–
4g	12.84	–	–	–	–
4h	1.61	0.83	0.18	3.53	12.00
4i	3.12	2.85	1.91	6.00	–
4j	0.32	0.38	0.88	–	–
4k	12.41	–	–	–	–
4l	1.49	0.33	1.42	–	–
4m	12.21	–	–	–	–
4n	0.072	0.50	–	–	–
4o	0.03	0.32	9.22	–	–
4p	0.22	0.42	12.32	–	–
5a	–	0.41	–	–	1.28
5b	–	0.60	–	–	0.67
6a	–	–	–	16.58	–
6b	–	–	–	–	3.10
6c	–	–	–	–	2.45
6d	–	–	–	–	4.82
6e	–	–	–	–	16.40
Standard[a]	0.005	0.008	0.004	0.008	0.001

All activities ≥ 100 μM were considered to be inactive indicated by (–).
[a] Standard used was Taxol (paclitaxel).

Promising cytotoxic activities were shown by flavones derivatives, **5a** and **5b** (0.41 μM–1.28 μM) against cell lines **PA1** and **KB403**, but were inactive against other cancer cell lines. Importantly, isoxazole hybrids, **6b**–**6e** selectively inhibited oral and mouth cancer cell line **KB403**, where **6c** (IC50 = 2.45 μM) was found to be most active. Similarly isoxazole hybrid **6a** (IC50 = 16.58 μM) was found to be selectivity cytotoxic for colon cancer cells **CaCO2**.

3. Conclusion

Novel hybrids of marine bromopyrrole alkaloids with chalcone, isoxazole and flavone structural feature were synthesized and evaluated for anticancer activity. Promising cytoxicity exhibited by these hybrids indicated that introduction of 4,5-dibromopyrrole feature into chalcones, isoxazoles and flavones improved their anticancer potential. Thus, these hybrids can act as leads for development of newer anticancer agent with improved potency and selectivity.

4. Experimental protocols

4.1. General

Ethanol and ACN (Acetonitrile) were freshly distilled from $CaCl_2$. All chromatographic solvents were distilled before use. Silica gel of 60–120 mesh and 200–400 mesh were obtained for column and flash chromatography. Some of the starting materials were

obtained from S.D. Fine Pvt. Ltd., SRL, Spectrochem, Aldrich and some of them were prepared in laboratory and used without further purification. All melting points (MP) were recorded on Thermomik Compbell electronics, having oil-heating system and were uncorrected. Analytical Thin-layer Chromatography (TLC) was carried out on precoated plates SiO_2 (silica gel 60, F 254, Merck). FTIR spectra were recorded on Perkin Elmer RX I spectrometer using KBr pellets. All the ^{1}H NMR spectra were recorded on JEOL AL-300 FT-NMR spectrometer with DMSO-d_6 as solvent using tetramethyl silane (TMS) as internal reference and ^{13}C (100 MHz) NMR were recorded on Varian Unity-400 Spectrometer. Mass spectra were obtained on THERMO FINNINGAN LCQ advantage max (LCMS).

4.2. General synthetic procedure for the target compounds (**4a**–**4p**)

In a round bottom flask equipped with sealed mechanical stirrer, 40% sodium hydroxide solution and 5–10 mL methanol were stirred in an ice-bath. Then **3a**/**3b** (1 mol) and substituted acetophenones (1 mol) were added to above mixture and stirred for half an hour. The reaction mixture was then refluxed on water bath till the yellow precipitate occurred. The reaction mixture was concentrated under reduced pressure and to the residue dilute acetic acid was added slowly till precipitation is complete. The solid precipitated was filtered by vacuum and dried. The obtained solid was recrystallized using ethanol.

4.2.1. 3-(4,5-Dibromo-1-methyl-pyrrol-2-yl)-1-(2-hydroxyphenyl) prop-2-en-1-one (**4a**)

Yield: 60%; m.p.; 170–172 °C; IR (KBr) V_{max} cm^{-1}: 1620 (C═C), 1715 (C═O), 3038 (C═C–H aromatic), 3440 (OH phenol); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.59 (s, 3H pyrrole N–CH_3), 5.3 (s, 1H phenol OH), 6.42 (s, 1H pyrrole 3H), 6.7–7.4 (m, 4H ArH), 7.78 (d, 1H, –COCH═, J = 15.3 Hz), 7.86 (d, 1H, ═CH–Ar, J = 15.3 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 192.3, 163.2, 135.6, 135.3, 129.5, 127.5, 121.6, 121.1, 117.9, 117.5, 100.2, 98.2, 27.8; Theoretical mass: 381.9072; MS m/z: 382.9152 (M+), 383.9231 (M + 2), 385.9158 (M + 4).

4.2.2. 3-(4,5-Dibromo-1-methyl-pyrrol-2-yl)-1-phenylprop-2-en-1-one (**4b**)

Yield: 56%; m.p.: 106–108 °C; IR (KBr) V_{max} cm^{-1}: 1622 (C═C), 1714 (C═O), 3049 (C═C–H aromatic); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.56 (s, 3H pyrrole N–CH_3), 6.47 (s, 1H pyrrole 3H), 6.9–7.5 (m, 4H ArH), 7.76 (d, 1H, –COCH═, J = 15.6 Hz), 7.82 (d, 2H, ═CH–Ar, J = 15.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.5, 137.2, 134.5, 133.6, 130.2, 129.6, 128.6, 125.3, 117.5, 100.3, 98.5, 27.9; Theoretical mass: 365.9130; MS m/z: 366.9210 (M+), 367.9289 (M + 2), 369.9442 (M + 4).

4.2.3. 3-(4,5-Dibromo-1-methyl-pyrrol-2-yl)-1-(1H-pyrrol-2-yl) prop-2-en-1-one (**4c**)

Yield: 62%; m.p.: 114–116 °C; IR (KBr) V_{max} cm^{-1}: 1621 (C═C), 1718 (C═O), 3058 (C═C–H aromatic); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.62 (s, 3H pyrrole N–CH_3), 6.42 (s, 1H pyrrole 3H), 6.9–7.56 (m, 3H pyrrole), 7.78 (d, 1H, –COCH═, J = 15.3 Hz), 7.83 (d, 2H, ═CH–Ar, J = 15.3 Hz), 12.36 (s, 1H pyrrole 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.2, 136.2, 133.2, 130.5, 129.5, 127.1, 126.5, 121.3, 119.6, 111.5, 98.6, 27.5; Theoretical mass: 354.9128; MS m/z: 355.9208 (M+), 356.9286 (M + 2), 358.9442 (M + 4).

4.2.4. 3-(4,5-Dibromo-1-methyl-pyrrol-2-yl)-1-(2,4-dimethoxyphenyl)prop-2-en-1-one (**4d**)

Yield: 58%; m.p.: 146–148 °C; IR (KBr) V_{max} cm^{-1}: 1624 (C═C), 1716 (C═O), 3035 (C═C–H aromatic); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.58 (s, 3H pyrrole N–CH_3), 3.86 (s, 6H OCH_3), 6.43 (s, 1H pyrrole 3H), 6.46–7.58 (m, 3H ArH), 7.63 (d, 1H, –COCH═, J = 15.0 Hz), 7.86 (d, 2H, ═CH–Ar, J = 15.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 190.2, 167.8, 162.3, 135.6, 131.2, 130.2, 128.5, 123.3, 118.5, 112.3, 101.3, 100.5, 98.6, 55.6, 55.6, 27.3; Theoretical mass: 425.9340; MS m/z: 426.9420 (M+), 427.9422 (M + 2), 429.9654 (M + 4).

4.2.5. 3-(4,5-Dibromo-1-methyl-pyrrol-2-yl)-1-(2,5-dimethoxyphenyl)prop-2-en-1-one (**4e**)

Yield: 52%; m.p.: 106–108 °C; IR (KBr) V_{max} cm^{-1}: 1632 (C═C), 1715 (C═O), 3048 (C═C–H aromatic); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.53 (s, 3H pyrrole N–CH_3), 3.84 (s, 6H OCH_3), 6.39 (s, 1H pyrrole 3H), 6.47–7.42 (m, 3H ArH), 7.66 (d, 1H, –COCH═, J = 15.6 Hz), 7.72 (d, 2H, ═CH–Ar, J = 15.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 190.3, 153.2, 149.6, 135.6, 130.2, 127.6, 121.2, 120.3, 117.9, 116.2, 103.2, 100.6, 98.6, 55.2, 55.2, 27.5; Theoretical mass: 425.9342; MS m/z: 426.9422 (M+), 427.9423 (M + 2), 429.9660 (M + 4).

4.2.6. 3-(4,5-Dibromo-1-methyl-pyrrol-2-yl)-1-(2,4-dichlorophenyl)prop-2-en-1-one (**4f**)

Yield: 65%; b.p.: 108 °C; IR (KBr) V_{max} cm^{-1}: 1625 (C═C), 1719 (C═O), 3052 (C═C–H aromatic); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.63 (s, 3H pyrrole N–CH_3), 6.40 (s, 1H pyrrole 3H), 6.72–7.58 (m, 3H ArH), 7.74 (d, 1H, –COCH═, J = 15.3 Hz), 7.82 (d, 2H, ═CH–Ar, J = 15.3 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.6, 145.2, 135.6, 135.2, 132.5, 131.6, 130.2, 129.6, 127.2, 126.5, 118.5, 100.5, 98.1, 27.9; Theoretical mass: 433.8355; MS m/z: 434.8435 (M+), 435.8436 (M + 2), 437.8673 (M + 4).

4.2.7. 3-(4,5-Dibromo-1-methyl-pyrrol-2-yl)-1-(3-hydroxyphenyl) prop-2-en-1-one (**4g**)

Yield: 68%; m.p.: 120–122 °C; IR (KBr) V_{max} cm^{-1}: 1620 (C═C), 1711 (C═O), 3045 (C═C–H aromatic), 3445 (OH phenol); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.55 (s, 3H pyrrole N–CH_3), 5.3 (s, 1H phenol OH), 6.37 (s, 1H pyrrole 3H), 6.6–7.42 (m, 4H ArH), 7.72 (d, 1H, –COCH═, J = 15.6 Hz), 7.86 (d, 2H, ═CH–Ar, J = 15.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.5, 165.2, 136.2, 132.5, 131.2, 130.2, 126.5, 123.6, 121.3, 118.6, 117.3, 100.6, 98.6, 27.5; Theoretical mass: 381.9075; MS m/z: 382.9155 (M+), 383.9158 (M + 2), 385.9392 (M + 4).

4.2.8. 3-(4,5-Dibromo-1-methyl-pyrrol-2-yl)-1-(2,4-dihydroxyphenyl)prop-2-en-1-one (**4h**)

Yield: 50%; m.p.: 118–120 °C; IR (KBr) V_{max} cm^{-1}: 1628 (C═C), 1712 (C═O), 3048 (C═C–H aromatic), 3442 (OH phenol); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.65 (s, 3H pyrrole N–CH_3), 5.35 (s, 2H phenol OH), 6.43 (s, 1H pyrrole 3H), 6.6–7.4 (m, 3H ArH), 7.63 (d, 1H, –COCH═, J = 15.0 Hz), 7.76 (d, 2H, ═CH–Ar, J = 15.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.5, 166.5, 164.3, 135.6, 132.9, 131.2, 127.8, 122.3, 114.3, 111.2, 104.6, 100.3, 98.6, 27.3; Theoretical mass: 397.9040; MS m/z: 398.9120 (M+), 399.9122 (M + 2), 401.9326 (M + 4).

4.2.9. 3-(4,5-Dibromo-1-methyl-pyrrol-2-yl)-1-(4-hydroxyphenyl) prop-2-en-1-one (**4i**)

Yield: 55%; m.p.: 122–124 °C; IR (KBr) V_{max} cm^{-1}: 1620 (C═C), 1715 (C═O), 3058 (C═C–H aromatic), 3448 (OH phenol); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.62 (s, 3H pyrrole N–CH_3), 5.36 (s, 1H phenol OH), 6.46 (s, 1H pyrrole 3H), 6.62–7.53 (m, 4H ArH), 7.64 (d, 1H, –COCH═, J = 15.3 Hz), 7.78 (d, 2H, ═CH–Ar, J = 15.3 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.5, 164.6, 135.6, 131.3, 131.3, 130.5, 130.2, 127.4, 118.6, 116.5, 116.5, 100.6, 98.6, 27.3; Theoretical mass: 381.9062; MS m/z: 382.9142 (M+), 383.9221 (M + 2), 385.9380 (M + 4).

*4.2.10. 3-(4,5-Dibromo-1-methyl-pyrrol-2-yl)-1-(2,5-dihydroxyphenyl)prop-2-en-1-one (**4j**)*

Yield: 55%; m.p.: 122–124 °C; IR (KBr) V_{max} cm^{-1}: 1622 (C=C), 1710 (C=O), 3078 (C=C–H aromatic), 3456 (OH phenol); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.68 (s, 3H pyrrole N–CH_3), 5.39 (s, 2H phenol OH), 6.39 (s, 1H pyrrole 3H), 6.69–7.48 (m, 3H ArH), 7.76 (d, 1H, –COCH=, J = 15.6 Hz), 7.82 (d, 2H, =CH–Ar, J = 15.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.2, 156.3, 151.2, 135.6, 130.2, 127.6, 123.6, 120.3, 120, 118.6, 107.6, 100.5, 98.1, 27.9; Theoretical mass: 397.9042; MS m/z: 398.9122 (M+), 399.9201 (M + 2), 401.9306 (M + 4).

*4.2.11. 1-(3-Aminophenyl)-3-(4,5-dibromo-1-methyl-pyrrol-2-yl) prop-2-en-1-one (**4k**)*

Yield: 58%; m.p.: 110–112 °C; IR (KBr) V_{max} cm^{-1}: 1620 (C=C), 1712 (C=O), 3062 (C=C–H aromatic), 3221 (NH_2); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.58 (s, 3H pyrrole N–CH_3), 5.86 (s, 2H NH), 6.40 (s, 1H pyrrole 3H), 6.7–7.58 (m, 4H ArH), 7.63 (d, 1H, –COCH=, J = 15.6 Hz), 7.67 (d, 2H, =CH–Ar, J = 15.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.6, 148.6, 138.6, 136.1, 134.5, 130.2, 128.6, 120.6, 118.7, 118.5, 114.2, 100.3, 98.6, 27.2; Theoretical mass: 380.9233; MS m/z: 381.9313 (M+), 382.9391 (M + 2), 384.9551 (M + 4).

*4.2.12. 3-(4,5-Dibromo-1H-pyrrol-2-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**4l**)*

Yield: 62%; m.p.: 98–100 °C; IR (KBr) V_{max} cm^{-1}: 1626 (C=C), 1716 (C=O), 3035 (C=C–H aromatic), 3440 (OH phenol); ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.35 (s, 1H phenol OH), 6.48 (s, 1H pyrrole 3H), 6.67–7.48 (m, 4H ArH), 7.76 (d, 1H, –COCH=, J = 15.3 Hz), 7.82 (d, 2H, =CH–Ar, J = 15.3 Hz), 12.6 (s, 1H pyrrole NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 190.6, 163.6, 139.6, 135.6, 129.8, 129.6, 128.3, 121.6, 121.4, 118.4, 100, 97.8, 97.2; Theoretical mass: 367.8925; MS m/z: 368.9005 (M+), 369.9083 (M + 2), 371.9241 (M + 4).

*4.2.13. 3-(4,5-Dibromo-1H-pyrrol-2-yl)-1-phenylprop-2-en-1-one (**4m**)*

Yield: 66%; m.p.: 116–118 °C; IR (KBr) V_{max} cm^{-1}: 1621 (C=C), 1716 (C=O), 3051 (C=C–H aromatic); ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.47 (s, 1H pyrrole 3H), 6.89–7.55 (m, 4H ArH), 7.64 (d, 1H, –COCH=, J = 15.3 Hz), 7.86 (d, 2H, =CH–Ar, J = 15.3 Hz), 12.3 (s, 1H pyrrole NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.5, 139.6, 137.2, 129.8, 129.2, 128.2, 127.5, 100, 97.8, 97; Theoretical mass: 365.9130; MS m/z: 366.9210 (M+), 367.9214 (M + 2), 369.9372 (M + 4).

*4.2.14. 3-(4,5-Dibromo-1H-pyrrol-2-yl)-1-(1H-pyrrol-2-yl)prop-2-en-1-one (**4n**)*

Yield: 66%; m.p.: 112–114 °C; IR (KBr) V_{max} cm^{-1}: 1623 (C=C), 1725 (C=O), 3052 (C=C–H aromatic); ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.43 (s, 1H pyrrole 3H), 6.72–7.56 (m, 3H pyrrole), 7.74 (d, 1H, –COCH=, J = 15.9 Hz), 7.80 (d, 2H, =CH–Ar, J = 15.9 Hz), 12.2 (s, 1H pyrrole NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.4, 139.2, 132.2, 130.5, 127.5, 127.1, 126.5, 111.5, 100.2, 97.6, 97.1; Theoretical mass: 340.8928; MS m/z: 341.9008 (M+), 342.9086 (M + 2), 344.9244 (M + 4).

*4.2.15. 3-(4,5-Dibromo-1H-pyrrol-2-yl)-1-(2,4-dimethoxyphenyl) prop-2-en-1-one (**4o**)*

Yield: 56%; m.p.: 142–144 °C; IR (KBr) V_{max} cm^{-1}: 1621 (C=C), 1718 (C=O), 3045 (C=C–H aromatic); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.84 (s, 6H OCH_3), 6.46 (s, 1H pyrrole 3H), 6.66–7.52 (m, 3H ArH), 7.72 (d, 1H, –COCH=, J = 15.0 Hz), 7.78 (d, 2H, =CH–Ar, J = 15.0 Hz), 12.26 (s, 1H pyrrole NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 192.2, 167.6, 162.2, 139.6, 131.2, 129.2, 127.5, 123.2, 112.3, 101.3, 100.3, 97.6, 96.9, 55.6; Theoretical mass: 411.9186; MS m/z: 412.9266 (M+), 413.9344 (M + 2), 415.9502 (M + 4).

*4.2.16. 3-(4,5-Dibromo-1H-pyrrol-2-yl)-1-(2,5-dimethoxyphenyl) prop-2-en-1-one (**4p**)*

Yield: 56%; m.p.: 116–118 °C; IR (KBr) V_{max} cm^{-1}: 1632 (C=C), 1713 (C=O), 3046 (C=C–H aromatic); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.86 (s, 6H OCH_3), 6.42 (s, 1H pyrrole 3H), 6.47–7.58 (m, 3H ArH), 7.66 (d, 1H, –COCH=, J = 15.3 Hz), 7.78 (d, 2H, =CH–Ar, J = 15.3 Hz), 12.3 (s, 1H pyrrole NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 192.3, 153.4, 150.6, 139.6, 129.6, 127.6, 122.2, 121.3, 115.9, 105.2, 100.2, 100, 98.6, 97, 55.8; Theoretical mass: 411.9186; MS m/z: 412.9266 (M+), 413.9344 (M + 2), 415.9502 (M + 4).

*4.3. General synthetic procedure for the target compounds (**5a** and **5b**)*

In a round bottom flask equipped with sealed mechanical stirrer and an efficient reflux condenser, **4a** (0.015 mol) or **4l** (0.015 mol) was stirred in dimethyl sulphoxide. To this mixture, Iodine (I_2, 0.015 mol) was added and reaction mixture was stirred for 30 min at 130 °C. The reaction mixture was treated with sodium thiosulphate (20% solution) and extracted with chloroform. Water was added to extracted liquid and then ethyl acetate. The organic layer containing product was passed through sodium sulphate and concentrated under reduced pressure. The obtained solid was recrystallized using ethanol.

*4.3.1. 2-(4,5-Dibromo-1H-pyrrol-2-yl)-4H-chromen-4-one (**5a**)*

Yield: 45.7%; b.p.: 136 °C; IR (KBr) V_{max} cm^{-1}: 1628 (C=C), 1718 (C=O), 3048 (C=C–H aromatic); ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.43 (s, 1H pyrrole 3H), 7.02 (s, 1H, chromen 3H), 7.55–7.99 (m, 4H ArH), 12.2 (s, 1H pyrrole NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.6, 168.2, 156.3, 135.6, 125.6, 123.6, 123.2, 118.5, 116.1, 110, 100.2, 97.6, 97; Theoretical mass: 365.8764; MS m/z: 366.8844 (M+), 367.8845 (M + 2), 369.8852 (M + 4).

*4.3.2. (4,5-Dibromo-1-methyl-pyrrol-2-yl)-4H-chromen-4-one (**5b**)*

Yield: 45.7%; b.p.: 136 °C; IR (KBr) V_{max} cm^{-1}: 1628 (C=C), 1718 (C=O), 3048 (C=C–H aromatic); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.58 (s, 3H pyrrole N–CH_3), 6.45 (s, 1H pyrrole 3H), 7.04 (s, 1H, chromen 3H), 7.5–8.0 (m, 4H ArH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.4, 159.3, 157.3, 135.4, 125.6, 125.3, 123.8, 123.4, 118.6, 118.4, 116.2, 106.6, 98.6, 29.9; Theoretical mass: 379.8918; MS m/z: 380.8998 (M+), 381.9076 (M + 2), 383.9234 (M + 4).

*4.4. General synthetic procedure for compounds (**6a**–**6e**)*

In a round bottom flask equipped with sealed mechanical stirrer and an efficient reflux condenser, 3-(4,5-dibromo-1-methyl-pyrrol-2-yl)-1-arylprop-2-en-1-one **4a**–**4c**, **4l** and **4n**, were dissolved in 20 mL ethanol. In a separate beaker, anhydrous sodium acetate (0.01 mol) was dissolved in minimum amount of hot acetic acid and this solution was then added to mixture of ammonium hydroxide hydrochloride (0.01 mol) in ethanol (15 mL). This mixture was added to solution of **4a**–**4c**, **4l** and **4n** in ethanol in a round bottom flask and refluxed on an oil-bath for 8 h. The reaction mixture was then concentrated under reduced pressure and neutralized with sodium hydroxide (0.1%). The obtained solid was dried and recrystallized using ethanol.

*4.4.1. 5-(4,5-Dibromo-1-methyl-pyrrol-2-yl)-3-phenylisoxazole (**6a**)*

Yield: 55%; m.p.: 118–120 °C; IR (KBr) V_{max} cm^{-1}: 1242 (C–O–C of isoxazole), 1628 (C=C), 3056 (C=C–H aromatic); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.68 (s, 3H pyrrole N–CH_3), 6.39 (s, 1H pyrrole 3H), 7.09 (s, 1H isoxazole 4H), 7.2–7.78 (m, 5H ArH); ^{13}C

NMR (100 MHz, DMSO-d_6): δ 163.3, 155.3, 150.3, 129.6, 129.6, 128.8, 128.3, 127.5, 127.5, 106.5, 105.3, 98.6, 98.3, 27.3; Theoretical mass: 378.9082; MS *m*/*z*: 379.9162 (M+), 380.9241 (M + 2), 382.9268 (M + 4).

*4.4.2. 5-(4,5-Dibromo-1-methyl-pyrrol-2-yl)-3-(1H-pyrrol-2-yl) isoxazole (**6b**)*

Yield: 68%; m.p.: 126–128 °C; IR (KBr) V_{max} cm^{-1}: 1246 (C–O–C of isoxazole), 1638 (C=C), 3046 (C=C–H aromatic); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.69 (s, 3H pyrrole N–CH_3), 6.43 (s, 1H pyrrole 3H), 7.10 (s, 1H isoxazole 4H), 7.22–7.68 (m, 3H pyrrole), 12.6 (s, 1H pyrrole NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 155.8, 150.6, 150.3, 129.6, 122.2, 111.2, 107.3, 106.9, 105.3, 98.7, 98.3, 27.6; Theoretical mass: 367.9032; MS *m*/*z*: 368.9112 (M+), 369.9116 (M + 2), 371.9274 (M + 4).

*4.4.3. 2-(5-(4,5-Dibromo-1-methyl-pyrrol-2-yl)isoxazol-3-yl) phenol (**6c**)*

Yield: 52%; m.p.: 136–138 °C; IR (KBr) V_{max} cm^{-1}: 1232 (C–O–C of isoxazole), 1642 (C=C), 3036 (C=C–H aromatic), 3420 (OH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.58 (s, 3H pyrrole N–CH_3), 5.35 (s, 1H phenol OH), 6.44 (s, 1H pyrrole 3H), 7.08 (s, 1H isoxazole 4H), 7.3–7.64 (m, 4H ArH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.5, 155.6, 155.3, 150.3, 131.6, 130.2, 121.6, 120.3, 117.6, 107.6, 105.6, 98.3, 98.1 27.6; Theoretical mass: 394.9025; MS *m*/*z*: 395.9105 (M+), 396.9106 (M + 2), 398.9342 (M + 4).

*4.4.4. 5-(4,5-Dibromo-1H-pyrrol-2-yl)-3-(1H-pyrrol-2-yl)isoxazole (**6d**)*

Yield: 58%; m.p.: 120–122 °C; IR (KBr) V_{max} cm^{-1}: 1238 (C–O–C of isoxazole), 1638 (C=C), 3046 (C=C–H aromatic); ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.43 (s, 1H pyrrole 3H), 7.09 (s, 1H isoxazole 4H), 7.22–7.68 (m, 2H pyrrole), 12.3 (s, 2H pyrrole NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 155.2, 150.2, 129.6, 128, 122.3, 111.2, 107.6, 106.2, 100.6, 98.5, 97.2; Theoretical mass: 353.8880; MS *m*/*z*: 354.8960 (M+), 355.9038 (M + 2), 357.9196 (M + 4).

*4.4.5. 2-(5-(4,5-Dibromo-1H-pyrrol-2-yl)isoxazol-3-yl)phenol (**6e**)*

Yield: 64%; m.p.: 128–130 °C; IR (KBr) V_{max} cm^{-1}: 1234 (C–O–C of isoxazole), 1642 (C=C), 3036 (C=C–H aromatic), 3424 (OH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.37 (s, 1H phenol OH), 6.47 (s, 1H pyrrole 3H), 7.06 (s, 1H isoxazole 4H), 7.4–7.62 (m, 4H ArH), 12.6 (s, 1H pyrrole NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 162, 155.4, 155.2, 131.6, 130.6, 128.9, 121.8, 120.5, 117.8, 107.6, 99.8, 97.1, 96; Theoretical mass: 380.8876; MS *m*/*z*: 381.8956 (M+), 382.9034 (M + 2), 384.9126 (M + 4).

4.5. Anticancer assay

Synthesized compounds were evaluated for in vitro cytotoxic activity against five human cancer cell lines **MCF7** (hormone dependant breast cancer cells), **PA1** (ovary cancer cells), **WRL68** (liver cancer cells), **CaCO2** (colon cancer cells) and **KB403** (oral and mouth cancer cells). Taxol (paclitaxel) was introduced as positive control in the assay. In vitro cytotoxicity testing was done using method by Woerdenbag et al. [32] In this method, 2×10^3 cells/well were incubated in the 5% CO_2 incubator for 24 h to enable them to adhere properly to the 96-well polystyrene microplate (Grenier, Germany). Test compounds dissolved in 100% DMSO (Merck, Germany), concentration less than 1.25%, in at least five doses, was added and left for 6 h after which the compound plus media was replaced with fresh media and the cells were incubated for another 48 h in the CO_2 incubator at 37 °C. After incubation, 10 μL MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma M 2128] was added in each well and plates were further incubated at 37 °C for 4 h which gave dark blue crystals after incubation period. In the last step, 100 μL of dimethyl sulfoxide (DMSO, Merck, Germany) was added and mixed thoroughly to dissolve the dark blue crystals. The plates were read normally on a SpectraMax 190 Microplate ELISA reader (Molecular Devices Inc. USA) at 570 nm within 1 h after addition of DMSO. The experiment was carried out in triplicate and the inhibitory concentration (IC) values were calculated by using following formula:

$$\%\text{inhibition} = [1 - \text{OD}(570\ \text{nm})\ \text{of sample well}/\text{OD}\ (570\ \text{nm})\ \text{of control well}] \times 100$$

% IC50 is the concentration in μg/mL required for 50% inhibition of cell growth as compared to that of untreated control.

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