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SYNTHESIS AND CYTOTOXIC EVALUATION OF SOME 2-{4-[(2-OXO-1,2-DIHYDRO-3H-INDOL-3-YLIDENE)METHYL] PHENOXY}-N-PHENYLACETAMIDE

VIVEK SINGH BHADAURIA[2], VISHNU SRAVANTHI[1], SUJEET KUMAR[1], DEBAJYOTI DAS[2], ERIK DE CLERCQ[3], DOMINIQUE SCHOLS[3] HARUKUNI TOKUDA[4] and SUBHAS S. KARKI[1]*,

[1]Department of Pharmaceutical Chemistry, KLE University's College of Pharmacy, Bangalore-560010, Karnataka, India
[2]Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Siksha 'O' Anusandhan University, Bhuvaneshawar, Orissa, India
[3]Rega Institute for Medical Research, KU Leuven, B-3000 Leuven, Belgium
[4] Department of Complementary and Alternative Medicine, R&D Graduate School of Medical Sciences, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-8640, Japan

Abstract: A series of 2-oxindole derivatives were synthesized and evaluated for cytotoxic activity against different human and murine cancer cell lines and cancer chemopreventive activity. Among the tested compounds **VS-06, 08, 12** and **17** displayed cytotoxic activity in the range of 5.0 to 8.5 μM against human T-lymphocyte cells (CEM). Results showed that molecules with electron withdrawing substituent at 4 position of N-phenylacetamide group exhibited an increase in activity against the human tumor cell line CEM. The cancer chemopreventive effect of **VS-01** (IC_{50} = 451 nM) displayed equipotent activity in comparison to standard oleanolic acid (IC_{50} = 449 nM).

Keywords: 2-oxindole derivatives, cytostatic activity, chemoprevention

Abbreviations: EBV-EA - Epstein–Barr virus early antigen, TPA - 12-*O*-tetradecanoylphorbol-13-acetate

Isatins (1H-indole-2,3-dione) are synthetically versatile substrates, which can be used for the synthesis of a large variety of heterocyclic compounds, such as indoles and quinolines, and as a starting material for drug synthesis. Isatins have also been found in mammalian tissue and their function as a modulator of biochemical processes has been described in the literature.

Hesperidin (I) (1, 2) and indirubin (II) (3) are natural alkaloids containing the 2-oxindole unit, and they exhibit antiangiogenic and anticancer activity, respectively (1, 2). Analogues of indirubin were found to act as inhibitors of cyclic-dependent kinases (CDKs) (3). Many 2-oxindole analogues have been evaluated for kinase inhibitory activities, like sunitinib (SU11248) (III) (4, 5), SU4984 (IV) (6-11) and GW491619 (V) (12). BIBF1000 and BIBF1120 (nintedanib) are 6-methoxycarbonyl group-5-substituted-2-oxindoles and are potent inhibitors of VEGFR-1/2/3, PDGFR and FGFR-1 with low cross-reactivity against other kinases (13). Notably, nintedanib is currently used for the treatment of idiopathic pulmonary fibrosis (IPF) and along with other medications for some types of non-small-cell lung cancer.

Recently, Wang et al., in 2015, synthesized 5-(5-halogenated-2-oxo-1*H*-pyrrolo[2,3-*b*]pyridin-(3*Z*)-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxamides (VI) as antitumor agents (14). Thus, a series 5-substituted-2-oxindole derivatives were designed, synthesized and evaluated for their cytotoxic and cancer chemoprevention activity.

EXPERIMENTAL

Chemistry

The melting points were taken in open capillary method and are uncorrected. Silica gel G plates

* Corresponding author: e-mail: subhasskarki@gmail.com; phone: +91 80 23325611; fax: +91 80 23425373

were used for TLC by using chloroform and methanol (95 : 5, v/v), spots were visualized in iodine chamber. The IR spectra were recorded in KBr discs on a Jasco 430+ (Jasco Japan) apparatus; the ^{1}H NMR spectra were recorded in $CDCl_3$/DMSO on a Bruker (400 MHz) (Bruker, Germany), and *J* values were reported in hertz (Hz). Mass spectra were recorded in triple quadrapole LCMS-6410 from Agilent Technologies. CHN analysis was recorded in Flash 2000 CHNS/O analyzer from Thermo Scientific (USA). Required formyl intermediates like 2-(4-formylphenoxy)-*N*-(4-nitrophenyl) acetamide, *N*-(4-chlorophenyl)-2-(4-formylphenoxy)acetamide, 2-(4-formylphenoxy)-*N*-(4-methylphenyl)acetamide, *N*-(4-chloro-3-fluoro -phenyl)-2-(4-formylphenoxy)acetamide, *N*-cyclohexyl-2-(4-formylphenoxy)acetamide (15) and 2-(4-formylphenoxy)-*N*-phenylacetamide (16) were prepared according to the literature. Solutions of the examined compounds were prepared in DMSO.

General procedure for the preparation of 2-{4-[(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)methyl]phenoxy}-*N*-arylacetamide (VS-01 to VS-18)

Equimolar quantity of 5-substituted-1H-indolin-2-ones and 2-(4-formylphenoxy)-N-substituted-phenyl-acetamide was taken into round bottom flask containing 50 mL of methanol, followed by 2-3 drops of piperidine. The reaction mixture was refluxed for 1-4 h or till the end of reaction as confirmed by TLC. Then, it was cooled to room temperature and, the solution was filtered. The obtained residue was recrystallized by using dimethylformamide and ethanol.

2-{4-[(2-Oxo-1,2-dihydro-3H-indol-3-ylidene) methyl]phenoxy}-N-phenylacetamide VS-01

Yield 65%, IR (KBr, cm^{-1}): 3349 (N-H), 3067 (C-H), 2841 (C-H), 1689 (C=O), 1667 (C=C), 1367 (C=N), 1057 (C-O). ^{1}H-NMR (δ, ppm): 10.56 (s, 1H, NH), 10.11 (s, 1H, NH), 8.48 (d, 2H, *J* = 8 Hz), 7.74 (s, 1H), 7.67-7.62 (m, 3H), 7.32 (t, 2H, *J* = 16), 7.17 (t, 1H, *J* = 16 Hz), 7.10 (d, 3H, *J* = 8 Hz), 6.97 (t, 1H, *J* = 16 Hz), 6.81 (s, 1H), 4.80 (s, 2H, -OCH_2-). MS (ESI) m/z: 369.00 (370.13). Analysis: calcd. for $C_{23}H_{18}N_2O_3$: C 74.58; H 4.90; N 7.56%; found: C 74.51; H 4.85; N 7.61%.

N-(4-nitrophenyl)-2-{4-[(2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]phenoxy} acetamide VS-02

Yield 62%, IR (KBr, cm^{-1}): 3385 (N-H), 3069 (C-H), 2933 (C-H), 1693 (C=O), 1604 (C=C), 1505 (NO_2), 1341 (C=N), 1059 (C-O). ^{1}H-NMR (δ, ppm): 10.74 (s, 1H, NH), 10.56 (s, 1H, NH), 8.47 (d, 2H, *J* = 8 Hz), 8.25 (d, 2H, *J* = 8 Hz), 7.92 (d, 2H, *J* = 8 Hz), 7.74 (s, 1H), 7.67 (d, 1H, *J* = 8 Hz), 7.17 (t, 1H, *J* = 16 Hz), 7.10 (d, 2H, *J* = 8 Hz), 6.97 (t, 1H, *J* = 16 Hz), 6.81 (d, 1H, *J* = 8 Hz), 4.89 (s, 2H, -OCH_2-). MS (ESI) m/z: 414.10 (415.12). Analysis: calcd. for $C_{23}H_{17}N_3O_5$: C 66.50; H 4.12; N 10.12%; found: C 66.45; H 4.15; N 10.15%.

N-(4-methylphenyl)-2-{4-[(2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]phenoxy} acetamide VS-03

Yield 64%, IR (KBr, cm^{-1}): 3346 (N-H), 3108 (ArC-H), 2916 (AlkC-H), 1665 (C=O), 1610 (C=C), 1306 (C=N), 1076 (C-O). ^{1}H-NMR (δ, ppm): 10.55 (s, 1H, NH), 10.02 (s, 1H, NH), 8.47 (d, 1H, *J* = 8 Hz), 7.74-7.71 (m, 1H), 7.67-7.62 (m, 1H), 7.52 (d, 2H, *J* = 8 Hz), 7.23-7.17 (m, 1H), 7.13 (t, 2H, *J* = 16 Hz), 7.09 (d, 1H, *J* = 8 Hz), 6.97 (t, 1H, *J* = 16 Hz), 6.87-6.84 (m, 1H), 6.81 (d, 1H, *J* = 8 Hz), 4.77 (s, 2H, -OCH_2-), 2.25 (s, 3H, CH_3). MS (ESI) m/z: 385.00 (384.43). Analysis: calcd. for $C_{24}H_{20}N_2O_3$: C 74.98; H 5.24; N 7.29%; found: C 74.85; H 5.15; N 7.35%.

N-(4-chlorophenyl)-2-{4-[(2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]phenoxy} acetamide VS-04

Yield 67%, IR (KBr, cm^{-1}): 3350 (N-H), 3071 (ArC-H), 2946 (AlkC-H), 1659 (C=O), 1615 (C=C), 1304 (C=N), 1015 (C-O). ^{1}H-NMR (δ, ppm): 10.56 (s, 1H, NH), 10.23 (s, 1H, NH), 8.47 (d, 2H, *J* = 8 Hz), 7.74 (s, 1H), 7.68-7.65 (m, 3H), 7.38 (d, 2H, *J* = 8 Hz), 7.17 (t, 1H, *J* = 16 Hz), 7.09 (d, 2H, *J* = 8 Hz), 6.98 (t, 1H, *J* = 16 Hz), 6.81 (d, 1H, *J* = 8 Hz), 4.80 (s, 2H, -OCH_2-). MS (ESI) m/z: 405.00 (404.84). Analysis: calcd. for $C_{23}H_{17}ClN_2O_3$: C 68.23; H 4.23; N 6.92%; found: C 68.11; H 4.15; N 6.95%.

2-{4-[(5-Methyl-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]phenoxy}-N-phenyl -acetamide VS-05

Yield 62%, IR (KBr, cm^{-1}): 3352 (N-H), 3034 (C-H), 2911 (C-H), 1681 (C=O), 1597 (C=C), 1257 (C=N), 1062 (C-O). ^{1}H-NMR (δ, ppm): 10.44 (s, 1H, NH), 10.11 (s, 1H, NH), 8.47 (d, 2H, *J* = 8 Hz), 7.70 (s, 1H), 7.64 (d, 2H, *J* = 8 Hz), 7.49 (s, 1H), 7.32 (t, 2H, *J* = 16 Hz), 7.16 (d, 1H, *J* = 8 Hz), 7.09-7.07 (m, 2H), 6.99 (d, 1H, *J* = 8 Hz), 6.70 (d, 1H, *J* = 8 Hz), 4.79, (s, 2H, -OCH_2-), 2.08 (s, 3H, CH_3). Analysis: calcd. for $C_{24}H_{20}N_2O_3$: C 74.98; H 5.24; N 7.29%; found: C 74.88; H 5.21; N 7.32%.

N-(4-nitrophenyl)-2-{4-[(5-methyl-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl] phenoxy}acetamide VS-06

Yield 66%, IR (KBr, cm^{-1}): 3385 (N-H), 3076 (C-H), 2863 (C-H), 1695 (C=O), 1645 (C=C), 1600 (NO_2), 1336 (C=N), 1064 (C-O). ^{1}H-NMR (δ, ppm): 10.74 (s, 1H, NH), 10.45 (s, 1H, NH), 8.47 (d, 1H, *J* = 8 Hz), 8.25 (d, 2H, *J* = 8 Hz), 7.93-7.88 (m, 2H), 7.73-7.70 (m, 2H), 7.54-7.44 (m, 1H), 7.17 (d, 1H, *J* = 8 Hz), 7.10 (d, 1H, *J* = 8 Hz), 7.04-6.97 (m, 1H), 6.76-6.67 (dd, 1H, *J* = 8.8 Hz), 4.89 (s, 2H, -OCH_2-), 2.16 (s, 3H, CH_3). MS (ESI) m/z: 430.00 (429.42). Analysis: calcd. for $C_{24}H_{19}N_3O_5$: C 67.13; H 4.46; N 9.79%; found: C 67.05; H 4.39; N 9.85%.

N-(4-methylphenyl)-2-{4-[(5-methyl-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]-phenoxy}acetamide VS-07

Yield 68%, IR (KBr, cm^{-1}): 3355 (N-H), 3069 (C-H), 2912 (C-H), 1692 (C=O), 1608 (C=C), 1315 (C=N), 1024 (C-O). ^{1}H-NMR (δ, ppm): 10.42 (s, 1H, NH), 10.02 (s, 1H, NH), 7.72 (d, 2H, *J* = 8 Hz), 7.54-7.51 (m, 3H), 7.45 (s, 1H), 7.13 (t, 4H, *J* = 16 Hz), 7.04 (d, 1H, *J* = 8 Hz), 6.76 (d, 1H, *J* = 8 Hz), 4.78 (s, 2H, -OCH_2-), 2.25 (s, 3H, -CH_3), 2.17 (s, 3H, -CH_3). MS (ESI) m/z: 399.00 (398.45). Analysis: calcd. for $C_{25}H_{22}N_2O_3$: C 75.36; H 5.57; N 7.03%; found: C 75.21; H 5.41; N 7.11%.

N-(4-chlorophenyl)-2-{4-[(5-methyl-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl] phenoxy}acetamide VS-08

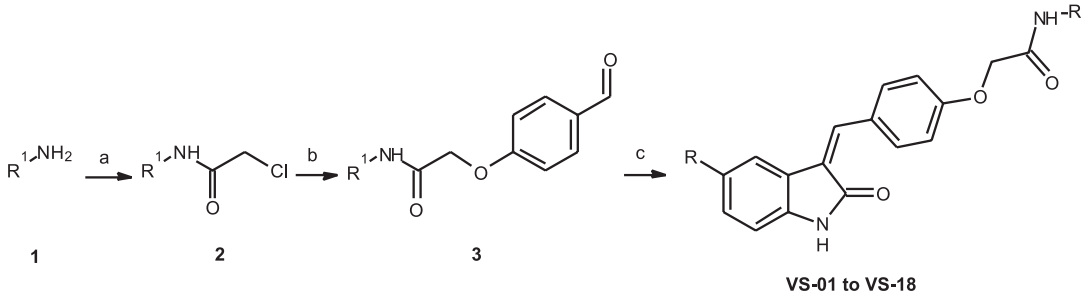
Yield 69%, IR (KBr, cm^{-1}): 3354 (N-H), 3093 (C-H), 3063 (C-H), 1693 (C=O), 1607 (C=C), 1316 (C=N), 1019 (C-O). ^{1}H-NMR (δ, ppm): 10.42 (s, 1H, NH), 10.26 (s, 1H, NH), 7.72-7.67 (dd, 4H, *J* = 8,8 Hz), 7.54 (s, 1H), 7.44 (s, 1H), 7.39 (d, 2H, *J* = 8 Hz), 7.16 (d, 2H, *J* = 8 Hz), 7.03 (d, 1H, *J* = 8 Hz), 6.76 (d, 1H, *J* = 8 Hz), 4.81 (s, 2H, -OCH_2-), 2.16 (s, 3H, CH_3). MS (ESI) m/z: 419.00 (418.87). Analysis: calcd. for $C_{24}H_{19}ClN_2O_3$: C 68.82; H 4.57; N 6.69%; found: C 68.75; H 4.52; N 6.75%.

2-{4-[(5-Chloro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]phenoxy}-N-phenyl acetamide VS-09

Yield 70%, IR (KBr, cm^{-1}): 3059 (C-H), 2852 (C-H), 1695 (C=O), 1596 (C=C), 1313 (C=N), 1059 (C-O). ^{1}H-NMR (δ, ppm): 10.69 (s, 1H, NH), 10.12 (s, 1H, NH), 8.50 (d, 2H, *J* = 8 Hz), 7.88 (s, 1H), 7.80 (s, 1H), 7.72 (d, 1H, *J* = 8 Hz), 7.66-7.62 (m, 2H), 7.30 (t, 2H, *J* = 16 Hz), 7.21-7.16 (m, 1H), 7.11-7.06 (m, 2H), 6.82 (d, 1H, *J* = 8 Hz), 4.81 (s, 2H, -OCH_2-). MS (ESI) m/z: 404.90 (404.85). Analysis: calcd. for $C_{23}H_{17}ClN_2O_3$: C 68.23; H 4.23; N 6.92%; found: C 68.18; H 4.19; N 6.99%.

2-{4-[(5-Chloro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]phenoxy}-N-(4-chloro phenyl)acetamide VS-12

Yield 71%, IR (KBr, cm^{-1}): 3378 (N-H), 3121 (C-H), 2854 (C-H), 1688 (C=O), 1660 (C=C), 1303 (C=N), 1011 (C-O). ^{1}H-NMR (δ, ppm): 10.69 (s, 1H, NH), 10.26 (s, 1H, NH), 8.49 (d, 2H, *J* = 8 Hz), 7.88 (s, 1H), 7.80 (s, 1H), 7.68 (d, 2H, *J* = 8 Hz), 7.39 (d, 2H, *J* = 8 Hz), 7.21-7.19 (m, 1H), 7.11 (d, 2H, *J* = 8 Hz), 6.82 (d, 1H, *J* = 8 Hz), 4.81(s 2H, -OCH_2-). MS (ESI) m/z: 386.1 (384.3). MS (ESI) m/z: 437.10 (439.05). Analysis: calcd. for $C_{23}H_{16}Cl_2N_2O_3$: C 62.88; H 3.67; N 6.38%; found: C 68.71; H 3.65; N 6.42%.



Where, R^1 = C_6H_5, 4-NO_2-C_6H_4, 4-CH_3-C_6H_4, 4-Cl-C_6H_4, 3-Cl-4-F-C_6H_3, C_6H_{11}

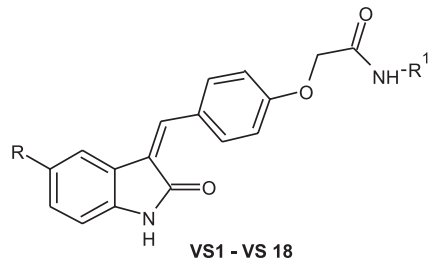
R= H, CH_3, Cl

Reagents: a) $ClCH_2COCl$, $(C_2H_5)_3N$, CH_2Cl_2, b) KI, K_2CO_3, 4-OH-C_6H_4-CHO, reflux 20 hrs

c) 2-oxindole, piperidine, CH_3OH, reflux 4hrs

Scheme 1. Synthesis of compounds **VS-01**-**VS-18**

Table 1. Physicochemical properties of synthetized compounds.



VS1 - VS 18

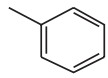
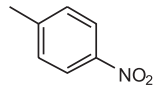
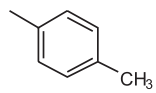
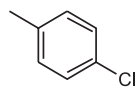
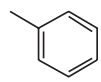
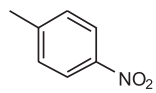
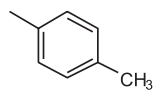
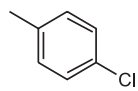
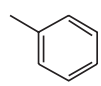
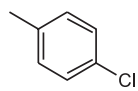
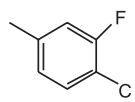
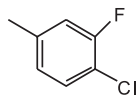
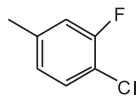
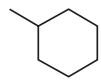
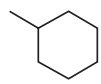
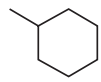
CODE	R	R[1]	Mol. formula	Mol. Wt.	M.P. °C	Rf* value
VS-01	H		$C_{23}H_{18}N_2O_3$	370.13	252-254	0.80
VS-02	H		$C_{23}H_{17}N_3O_5$	415.12	296-298	0.78
VS-03	H		$C_{24}H_{20}N_2O_3$	384.43	270-273	0.74
VS-04	H		$C_{23}H_{17}ClN_2O_3$	404.85	274-276	0.76
VS-05	CH_3		$C_{24}H_{20}N_2O_3$	384.43	248-250	0.78
VS-06	CH_3		$C_{24}H_{19}N_3O_5$	429.42	230-232	0.76
VS-07	CH_3		$C_{25}H_{22}N_2O_3$	398.45	240-242	0.74
VS-08	CH_3		$C_{24}H_{19}ClN_2O_3$	418.87	252-254	0.68
VS-09	Cl		$C_{23}H_{17}ClN_2O_3$	404.85	268-270	0.74
VS-12	Cl		$C_{23}H_{16}Cl_2N_2O_3$	439.05	252-254	0.72
VS-13	H		$C_{23}H_{16}ClFN_2O_3$	422.84	220-223	0.70

Table 1. Cont.

CODE	R	R^1	Mol. formula	Mol. Wt.	M.P. ^oC	Rf* value
VS-14	CH_3		$C_{24}H_{18}ClFN_2O_3$	436.86	248-250	0.72
VS-15	Cl		$C_{23}H_{15}ClFN_2O_3$	457.28	275-276	0.72
VS-16	H		$C_{23}H_{24}N_2O_3$	376.45	240-242	0.78
VS-17	CH_3		$C_{24}H_{26}N_2O_3$	390.47	264-266	0.72
VS-18	Cl		$C_{23}H_{23}N_2O_3$	410.89	278-280	0.76

* Mobile phase:- $CHCl_3$-CH_3OH (9.5 : 0.5, v/v)

N-(4-chloro-3-fluorophenyl)-2-{4-[(2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl] phenoxy}acetamide VS-13

Yield 69%, IR (KBr, cm^{-1}): 3437 (N-H), 3074 (C-H), 2908 (C-H), 1691 (C=O), 1613 (C=C), 1321 (C=N), 1067 (C-O). ^{1}H-NMR (δ, ppm): 10.58 (s, 1H, NH), 10.35 (s, 1H, NH), 8.49 (d, 2H, *J* = 8 Hz), 7.97-7.94 (m, 1H), 7.76-7.72 (m, 1H), 7.68 (d, 1H, *J* = 8 Hz), 7.60-7.56 (m, 1H), 7.42 (t, 1H, *J* = 16 Hz), 7.24-7.21 (m, 1H), 7.19 (d, 2H, *J* = 8 Hz), 6.98 (t, 1H, *J* = 8 Hz), 6.82 (d, 1H, *J* = 8 Hz), 4.82 (s, 2H, -OCH_2-). MS (ESI) m/z: 423.00 (422.84). Analysis: calcd. for $C_{23}H_{16}ClFN_2O_3$: C 65.33; H 3.81; N 6.63%; found: C 65.26; H 3.74; N 6.65%.

N-(4-chloro-3-fluorophenyl)-2-{4-[(5-methyl-2-oxo-1,2-dihydro-3H-indol-3-ylidene) methyl]phenoxy}acetamide VS-14

Yield 66%, IR (KBr, cm^{-1}): 3400 (N-H), 3076 (C-H), 2919 (C-H), 1694 (C=O), 1616 (C=C), 1246 (C=N), 1068 (C-O). ^{1}H-NMR (δ, ppm): 10.47 (s, 1H, NH), 10.34 (s, 1H, NH), 8.49 (d, 2H, *J* = 8 Hz), 7.97-7.95 (m, 1H), 7.71 (s, 1H), 7.60-7.56 (m, 1H), 7.50 (s, 1H), 7.40 (t, 1H, *J* = 16 Hz), 7.11 (d, 2H, *J* = 8 Hz), 7.01 (d, 1H, *J* = 8 Hz), 6.71 (d, 1H, *J* = 8 Hz), 4.82 (s, 2H, -OCH_2-), 2.30 (s, 3H, -CH_3). MS (ESI) m/z: 437.00 (436.86). Analysis: calcd. for $C_{24}H_{18}ClFN_2O_3$: C 65.98; H 4.15; N 6.41%; found: C 65.85; H 4.09; N 6.44%.

N-(4-chloro-3-fluorophenyl)-2-{4-[(5-chloro-2-oxo-1,2-dihydro-3H-indol-3-ylidene) methyl]phenoxy}acetamide VS-15

Yield 60%, IR (KBr, cm^{-1}): 3383 (N-H), 3178 (C-H), 2915 (C-H), 1691 (C=O), 1596 (C=C), 1309 (C=N), 1071 (C-O). MS (ESI) m/z: 457.00 (457.28). Analysis: calcd. for $C_{23}H_{15}ClFN_2O_3$: C 60.41; H 3.31; N 6.13%; found: C 60.55; H 3.26; N 6.19%.

N-cyclohexyl-2-{4-[(2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]phenoxy} acetamide VS-16

Yield 71%, IR (KBr, cm^{-1}): 3291.89 (N-H), 3091 (C-H), 2929 (C-H), 1709 (C=O), 1640 (C=C), 1331 (C=N), 1031 (C-O). ^{1}H-NMR (δ, ppm): 10.57 (s, 1H, NH), 7.99 (d, 1H, *J* = 8 Hz), 7.72 (d, 2H, *J* = 8 Hz), 7.63 (d, 1H, *J* = 8 Hz), 7.58 (s, 1H), 7.22 (t, 1H, *J* = 16 Hz), 7.10 (d, 2H, *J* = 8 Hz), 6.88 (d, 1H, *J* = 8 Hz), 4.56 (s, 2H, -OCH_2-), 3.62 (s, 1H, br, NH), 1.74-1.68 (m, 4H, alkyl), 1.58-1.23 (m, 7H, alkyl). MS (ESI) m/z: 377.00 (376.45). Analysis: calcd. for $C_{23}H_{24}N_2O_3$: C 73.38; H 6.43; N 7.44%; found: C 73.26; H 6.39; N 7.49%.

N-cyclohexyl-2-{4-[(5-methyl-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl] phenoxy}acetamide VS-17

Yield 69%, IR (KBr, cm^{-1}): 3345.89 (N-H), 3022 (C-H), 2931 (C-H), 1701 (C=O), 1653 (C=C), 1315 (C=N), 1028 (C-O). MS (ESI) m/z: 391.10 (390.47). Analysis: calcd. for $C_{24}H_{26}N_2O_3$: C 73.82; H 6.71; N 7.17%; found: C 73.85; H 6.66; N 7.20%.

N-cyclohexyl-2-{4-[(5-chloro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl] phenoxy}acetamide VS-18

Yield 66%, IR (KBr, cm^{-1}) 3418 (N-H), 3089 (C-H), 2930 (C-H), 1694 (C=O), 1656 (C=C), 1375 (C=N), 1065 (C-O). MS (ESI) m/z: 411.10 (410.89). Analysis: calcd. for $C_{23}H_{23}N_2O_3$: C 67.23; H 5.64; N 6.82%; found: C 67.15; H 5.58; N 6.90%.

BIOEVALUATIONS

Cytostatic activity

The methodology for performing the antiproliferative assays has been published previously (17). In brief, varying concentrations of the compounds (5-fold dilutions) were incubated at 37°C for 72 h or 48 h (L1210 cells) in 200 µL 96-well microtiter plates, and the viable tumor cell number was counted at the end of the incubation period using a Coulter counter (Coulter Electronics, Harpenden Hertz, U.K.).

Cancer chemoprevention activity-cells

EBV genome-carrying lymphoblastoid cells (Raji cells derived from Burkitt's lymphoma) were cultured in 10% fetal bovine serum (FBS) in RPMI-1640 under the conditions described previously (18). Spontaneous activation of EBV-EA in the sub-line of Raji cells was less than 0.1%.

Inhibition of EBV-EA activation assay

EBV-EA-positive serum from a patient with nasopharyngeal carcinoma (NPC) was a gift from the Department of Biochemistry, Oita Medicinal University. The EBV genome-carrying lymphoblastoid cells (Raji cells derived from Burkitt's lymphoma) were cultured in 10% fetal bovine serum (FBS) in RPMI-1640 medium (Nissui). Spontaneous activation of EBV-EA in our sub-line Raji cells was less than 0.1%. The inhibition of EBV-EA activation was assayed using Raji cells (virus non-producer type) as described previously (19). The indicator cells (Raji cells, 1-106/mL) were incubated at 37°C for 48 h in 1 mL of a medium con-

Table 2. Inhibitory effects of compounds on the proliferation of murine leukemia cells (L1210) and human T-lymphocyte cells (CEM) and human cervix carcinoma cells (HeLa).

Compound	IC_{50}* (µM)		
	L1210	CEM	HeLa
VS-01	≥ 250	≥ 250	16 ± 2
VS-02	41 ± 25	64 ± 54	19 ± 12
VS-03	> 250	> 250	> 250
VS-04	> 250	≥ 250	> 250
VS-05	> 250	≥ 250	> 250
VS-06	20 ± 9	5.1 ± 1.2	142 ± 83
VS-07	31 ± 7	22 ± 3	39 ± 28
VS-08	11 ± 4	5.0 ± 1.4	72 ± 16
VS-09	> 250	159 ± 44	> 250
VS-12	15 ± 4	8.5 ± 6.2	9.9 ± 8.7
VS-13	42 ± 20	21 ± 1	92 ± 11
VS-14	26 ± 10	15 ± 11	51 ± 17
VS-15	18 ± 2	11 ± 6	18 ± 2
VS-16	13 ± 9	13 ± 3	20 ± 4
VS-17	21 ± 1	5.2 ± 0.8	19 ± 3
VS-18	69 ± 43	21 ± 16	145 ± 72
Melphalan	2.13 ± 0.02	2.47 ± 0.21	NT

*50% inhibitory concentration. NT – not tested

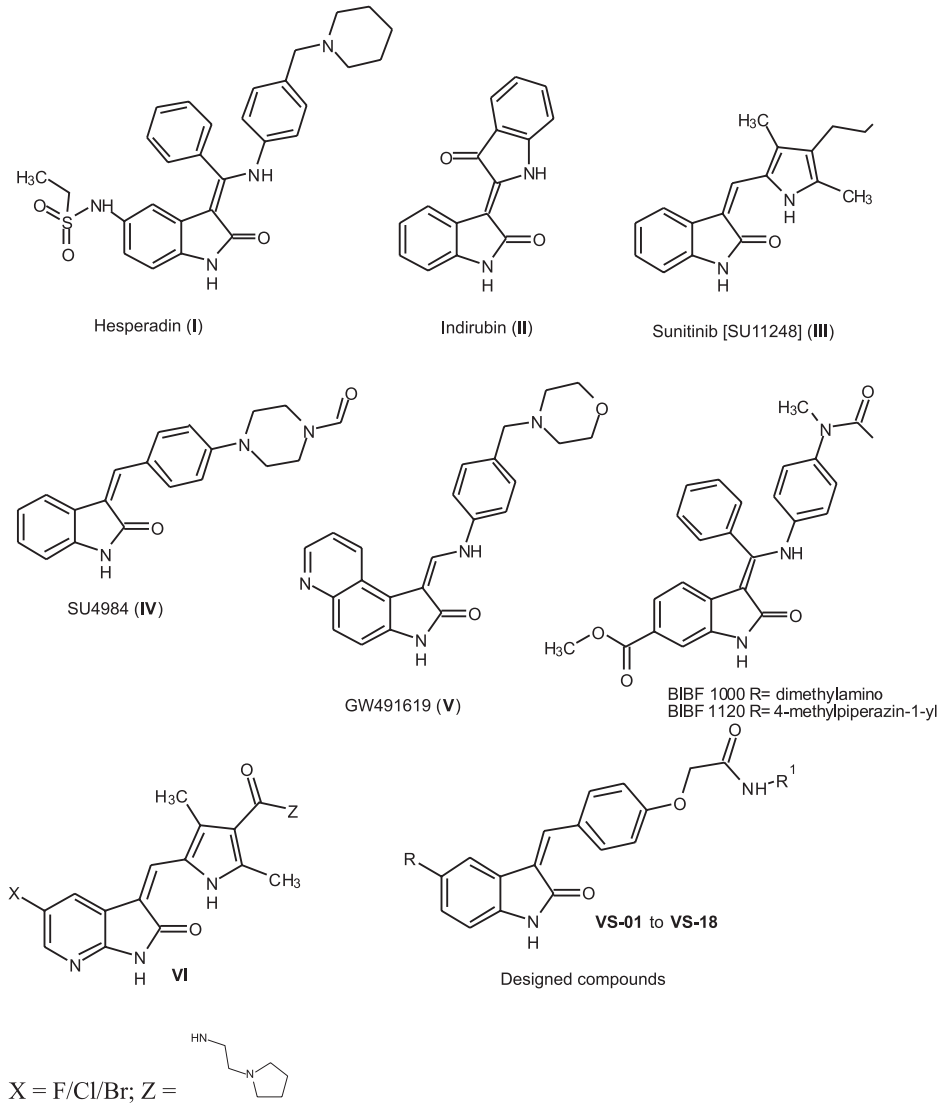


Figure 1. Some bioactive 2-oxindole derivatives

taining n-butyric acid (4 mmol), TPA (32 pmol = 20 ng in dimethyl sulfoxide (DMSO), 2 mL) as an inducer and various amounts of test compounds in 5 mL DMSO. Smears were made from the cell suspension, and the activated cells that were stained by EBV-EA-positive serum from NPC patients were detected by an indirect immunofluorescence technique (20). In each assay, at least 500 cells were counted, and the number of stained cells (positive cells) present was recorded. Triplicate assays were performed for each compound. The average EBV-EA induction of the test compounds was expressed as a relative ratio to the control experiment (100%) which was carried out only with n-butyric acid (4 mmol) plus TPA (32 pmol). EBV-EA induction was ordinarily around 35%. The viability of treated Raji cells was assayed by the Trypan Blue staining method.

RESULTS AND DISCUSSION

Chemistry

Compounds were synthesized *via* 3-step procedure (Scheme 1). Substituted amines **1** were reacted with chloroacetyl chloride in the presence of triethylamine to get **2** in good yield. Intermediate **3** was obtained by reacting 4-hydroxybenzaldehyde with **2** in the presence of potassium iodide and potassium carbonate by refluxing for 20 h. Title compounds (**VS-01** to **VS-18**) were obtained by reacting 2-oxindole with intermediate **3** in the presence of piperidine in methanol. Purity of all the synthesized compounds

was checked by TLC on precoated silica gel GF_{254} plates using chloroform and methanol as mobile phase. The structures of the synthesized compounds were confirmed by IR, NMR and mass spectrometry. Vibration of N-H bonds were observed between 3418-3292 cm^{-1} and for aromatic C-H bonds were observed between 3178-3022 cm^{-1}, whereas for aliphatic C-H bonds observed between 2946-2840 cm^{-1}, and for C=O bonds observed between 1709-1659 cm^{-1}. In proton NMR, the proton in N-H appeared between 10.80-10.01 ppm. All the synthesized compounds showed prominent signals for aromatic protons around 8.50-6.60 ppm. Protons of $-OCH_2-$ for the compounds appeared between 4.90-4.50 ppm and protons of $-CH_3$ appeared between 2.49-2.25 ppm. The structures of all the compounds were finally ascertained by mass spectra.

Bioevaluations

Cytotoxicity in human and murine tumor cell lines

The compounds were evaluated for their cytotoxic activity against human cervix carcinoma HeLa and CEM T-lymphocytes as well as murine L1210 leukemia cells. The data are summarized in Table 2 in comparison with melphalan. Several compounds (**VS-06**, **08**, **12** and **17**) showed IC_{50} values in the low micromolar range (5.0–8.5 µM) and were two to three times less potent than the standard melphalan (2.13–2.47 µM). Human T-lymphocytic CEM cells were more sensitive to the cytotoxic activity of the compounds in the series than L1210 and cervix carcinoma HeLa cells or murine L1210 leukemia cells. Compounds **VS-02, 06-08,** and **12-18** displayed mild cytotoxic activity (IC_{50} = 11.00-145.00 µM) against all three cell lines tested. Compound **VS-01** showed cytotoxic activity at 16 µM against human cervix carcinoma HeLa cells and was inactive against the other two cell lines namely L1210 and CEM tested. Compounds **VS-03** to **05** and **09** were inactive against all three cell lines tested.

The most potent cytotoxicity noted against T-lymphocytic cells (CEM) than the ;eukemia L1210 and cervix carcinoma HeLa cells. Replacement of hydrogen atom with CH_3 substituent at 5^{th} position of 2-oxindole led to a more potent inhibitors of tumor cell proliferation (**VS-05, 08** and **12**) against CEM cells. In a number of cases, replacement of the hydrogen at N-phenyl acatamide by an electron withdrawing group like NO_2 or Cl or 3-F-4-Cl

Table 3. Relative ratio[a] of EBV-EA activation levels (%) in the presence of compounds **VS-01** to **VS-18** and oleanolic acid.

	Concentration (mol ratio/ TPA)				IC_{50}
	1000	500	100	10	(nM)
VS-01	7.2 (60)[b]± 0.5	41.1 ± 1.6	73.8 ± 1.6	96.5 ± 1.5	451
VS-02	10.8 (60) ± 0.6	43.5 ± 1.2	76.3 ± 1.5	100 ± 1.0	480
VS-03	9.0 (60) ± 0.5	42.1 ± 1.4	74.8 ± 1.7	98.0 ± 1.3	475
VS-04	11.6 (60) ± 0.4	43.5 ± 1.3	75.9 ± 1.5	100 ± 1.0	492
VS-05	9.2(60) ± 0.5	41.9 ± 1.6	75.5 ± 1.7	100 ± 1.2	478
VS-06	12.7 (60) ± 0.4	44.8 ± 1.4	76.7 ± 1.5	100 ± 0.9	500
VS-07	10.0 (60) ± 0.4	44.2 ± 1.5	77.0 ± 1.4	100 ± 1.0	483
VS-08	12.5 (60) ± 0.3	45.1 ± 1.4	77.3 ± 1.5	100 ± 0.9	505
VS-09	11.8 (60) ± 0.4	44.1 ± 1.3	76.0 ± 1.5	100 ± 1.7	495
VS-12	13.3 (60) ± 0.5	46.8 ± 1.5	78.3 ± 1.6	100 ± 0.8	516
VS-13	13.5 (60) ± 0.4	46.5 ± 1.4	77.9 ± 1.6	100 ± 0.8	511
VS-14	14.0 (60) ± 0.4	47.9 ± 1.4	78.9 ± 1.6	100 ± 0.7	519
VS-15	15.3 (60) ± 0.6	47.5 ± 1.6	79.5 ± 1.4	100 ± 0.6	523
VS-16	8.5 (60) ± 0.4	41.6 ± 1.4	74.1 ± 1.6	98.7 ± 1.1	465
VS-17	11.5 (60) ± 0.4	42.0 ± 1.3	75.0 ± 1.5	100 ± 0.9	492
VS-18	14.8 (60) ± 0.5	48.7 ± 1.5	80.0 ± 1.5	100 ± 1.0	521
Oleanolic acid[c]	12.7 (70)	30.0	80.0	100	449

[a] The value obtained in the assay where the EBV-EA activation was performed by treatment with TPA (32 pmol) alone (without adding any 2-oxindole) was evaluated as 100%. [b] Values in parentheses are the percentage viability of Raji cells. [c] A standard sample to compare the inhibitory activities of **VS-01** to **VS-18** against EBV-EA activation.

resulted in a marked potentiation of the cytotoxic activity (compare **VS-01** with **02**; **VS-05** with **06, 08** & **12**; **VS-01** with **13-15**) (Table 2). In addition, replacement of an aryl ring at R_1 by cyclohexyl ring often resulted in significant improvement of cytotoxic activity; i.e., compare **VS-01** with **16**; **VS-05** with **17**; and **VS-09** with **18**.

Chemoprevention activity

The synthesized compounds (**VS-01** to **VS-18**) were subjected to *in vitro* inhibition assay against EBV-EA activation.

Tumor promoter activates EBV-EA, which produced viral early antigen (EA). The evaluation of its inhibitors is used as a primary screen for *in vivo* anti-tumor promoting activities (18). The *in vitro* inhibitory activities against EBV-EA activation are shown in Table 3 for the title compounds (**VS-01** to **VS-18**). To compare with the test compounds **VS-01** to **VS-18**, oleanolic acid was used as standard. The effect of test compounds on the viability of Raji cells and their 50% inhibitory concentration (IC_{50}) values have been shown in Table 3. The *in vitro* result of compound **VS-01** showed equipotent cytotoxicity against cell line tested. Its inhibitory activity was 451 mol ratio/32 pmol/TPA. The relative rates of **VS-01** with respect to TPA (100%) were 7.2, 41.1, 73.8, 96.5%, at concentrations of 1000, 500, 100 and 10 mol ratio/TPA (Table 3), showing 92.8, 58.9, 26.2, (compound **VS-01**) inhibition of TPA-induced EBV-EA activation whereas compounds **VS-16** (465 nM), **VS-03** (475 nM), **VS-05** (478 nM) and **VS-02** (480 nM) displayed moderate cytotoxicity. Compounds **VS-16**, **03**, **05** and **02** exhibited mild inhibitory activity.

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