

Synthesis and Cytotoxic Evaluation of Novel 3-Substituted Derivatives of 2-Indolinone

Shaya Mokhtari^{a,d}, Mahmoud Mosaddegh^b, Maryam Hamzeloo Moghadam^b,
Zohreh Soleymani^a, Saeideh Ghafari^b and Farzad Kobarfard^{a,c,e*}

^aDepartment of Medicinal Chemistry, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran. ^bTraditional Medicine and Materia Medica Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. ^cPharmaceutical Research Sciences Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. ^dStudent Research Committee, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran. ^ePhytochemistry Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Abstract

The assessment of the degree or rate of cellular proliferation and cell viability is critical for the assessment of the effects of drugs on both normal and malignant cell populations. In the present study, a few novel 3-substituted derivatives of 2-indolinones were synthesized by condensation of substituted oxindole or isatin derivatives with appropriate aldehydes or primary aromatic amines respectively. The synthesized compounds were screened for their cytotoxicity against HT-29 (human colon adenocarcinoma cell line) and MCF-7 (human breast adenocarcinoma cell line) cells using short term cytotoxicity MTT assay protocol. A few derivatives with $IC_{50} < 10 \mu M$ were identified among them. The compound bearing 5-bromo substitution was the most potent derivative. Global physicochemical properties for compounds IVa-e and Va-h were calculated and the two most active compounds (IVa and IVb) showed similar CLogP values.

Keywords: 3- substituted derivatives of 2-indolinones; Indole derivatives; Cytotoxicity; Colon and breast cancer; MTT assay.

Introduction

Cancer is a major health concern all around the world. Progresses in prevention and treatment of cancer have decreased the health rate, but the number of new diagnoses continues to increase. Therefore, new and more efficient anticancer agents are required to battle different cancer diseases. As a part of our efforts to find new chemotherapeutic agents as potential anticancer agent, we have synthesized a series of

indole derivatives. Indole derivatives represent many important classes of therapeutical agents in medicinal chemistry such as anti cancer (1), antioxidant (2), anti rheumatoid arthritis (3) and anti HIV (4, 5).

Some studies revealed that a group of 2-phenyl indole sulfamat are steroid sulfatase inhibitors with anti proliferative activity in breast cancer cells (6).

Some of the sulfur containing 2-phenyl indole analogues show *in-vivo* antineoplastic and anti estrogenic activity (7, 8).

The chemopreventive properties of cruciferus vegetables are attributed to the antitumor

* Corresponding author:

E-mail: kobarfard@sbumu.ac.ir

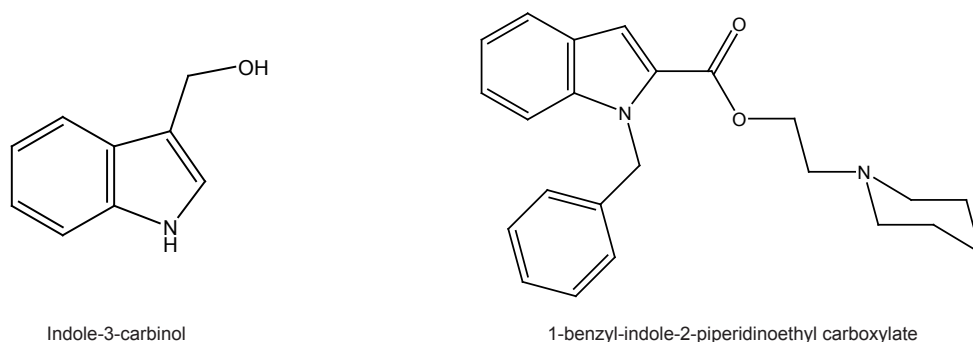


Figure 1. Two biologically effective indole structures.

activity of indole-3-carbinol (Figure 1) and its metabolic derivatives, which have shown great potential for both prevention and treatment of cancer through numerous mechanisms such as induction of apoptosis and cell cycle arrest, antiestrogenic activity, gene expression modulation and prevention of carcinogen-DNA adduct formation (9,10).

Olgen *et al.* have viewed indole ring as heterocyclic ATP analogue and discovered a few new indole derivatives with tyrosine kinase inhibitory activity. They have reported that 1-benzyl-indole-2-piperidinoethyl carboxylate is a potent inhibitor of Src tyrosine kinase with IC_{50} of 1.34 μ M (11) (Figure 1).

They also introduced a series of 3-(substituted-benzylidene)-1,3-dihydro-indolin-2-thione derivatives and their corresponding indolin-2-one congeners and evaluated their ability to inhibit Src PTK. In this study, (Z)-3-(4'-dimethylaminobenzylidene)-1,3-dihydro-indolin-2-thion (II) and (Z)-3-(2',6'-dichlorobenzylidene)-1,3dihydro-indolin-2-thion (III), were identified as moderately active Src PTK inhibitors with IC_{50} of 21.91 and

21.20 μ M respectively (12) (Figure 2).

In an effort to find novel indole-based compounds with potential anticancer activity, a few 3-benzylidene indole-2-one and 3-phenylimino indole-2-one derivatives were synthesized and evaluated for their cytotoxic activity against HT-29 (human colon adenocarcinoma cell line) and MCF7 (human breast adenocarcinoma cell line) using short term cytotoxicity MTT assay protocol. It is proven that tyrosine kinases of the Src family (SFK) are frequently deregulated in human colorectal cancer (CRC) (13). The overexpression of tyrosine kinases in high percentages in human breast cancers is also well documented (14).

A series of thirteen 3-benzylidene indole-2-ones and 3-phenyliminoindole-2-ones (IV_{a-c} and V_{a-h}) were prepared in our lab as shown in (Table 1). These compounds were screened for their cytotoxic activities against colon (HT29) and breast (MCF7) cancer cell lines.

Compounds IV_{a-c} were synthesized by condensation of appropriate indole-2-one with different aromatic aldehydes in the presence of

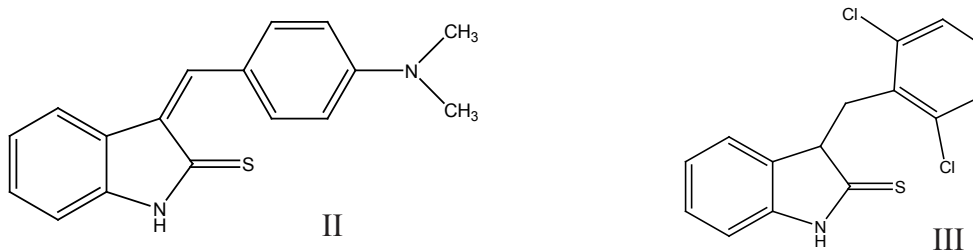


Figure 2. Chemical structure of (II) and (III) which are moderately active Src PTK inhibitor.

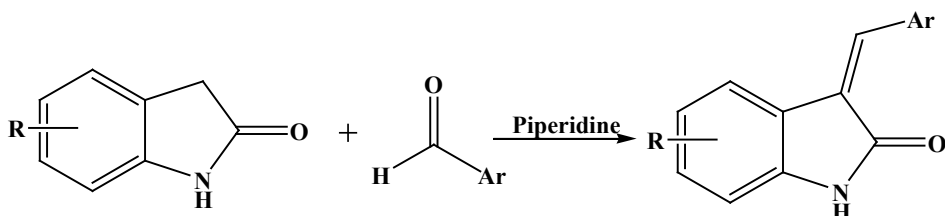


Figure 3. Compounds IV_{a-e} synthesis scheme.

piperidine as base (Figure 3).

In the case of compound IV_c the aldehyde was synthesized in three steps starting from 4-(bromomethyl)benzocnitrile (Figure 4).

Compounds V_{a-h} were synthesized by condensation of the appropriate isatin derivatives with the proper aromatic amines in the presence of acetic acid (Figure 5).

Experimental

Chemistry

All solvents, reagents and catalysts were of analytical grade and used without further purification. The melting points (°C) were determined by open capillary method on an Electrothermal melting point apparatus and

were uncorrected. The purity of compounds was confirmed by thin layer chromatography using Whatman Sil G/UV₂₅₄ silica gel plates as the stationary phase and with suitable mobile phase with fluorescent indicator, and the spots were visualized under 254 and 366 nm illumination. Infrared spectra were recorded as thin films on KBr plates with ν_{\max} in inverse centimeters. ¹H NMR spectra were recorded on a Bruker DRX-Avance (500 MHz) and or (250 MHz) spectrometer using DMSO-d₆ and CDCl₃ as solvents and Chemical shift values are expressed in ppm (parts per million) relative to tetramethylsilane (TMS) as internal standard; s = singlet, d = doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet, brs = broad singlet. Mass analyses were performed

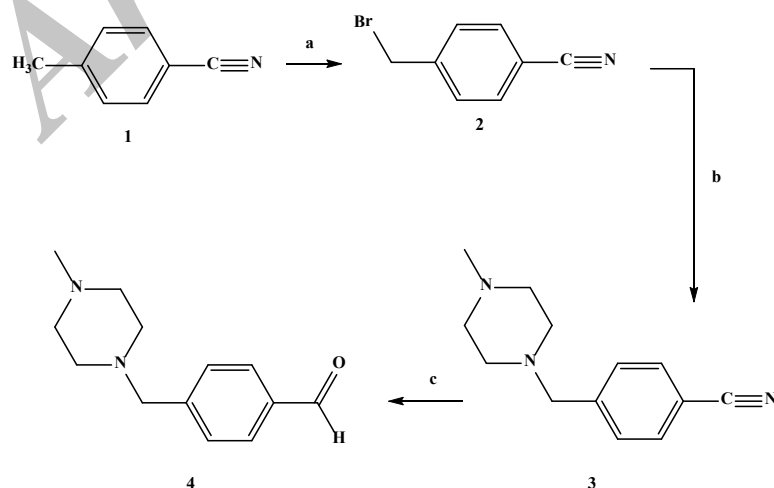
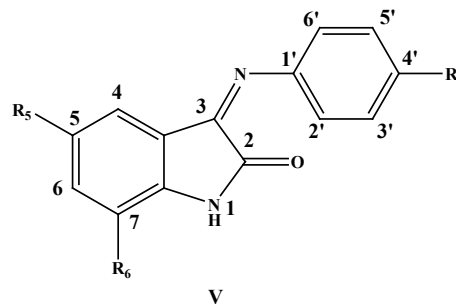
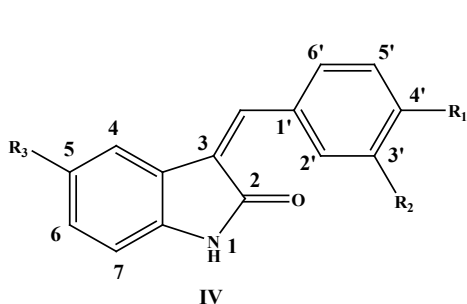


Figure 4. Synthesis of 4-((4-methylpiperazin-1-yl) methyl)benzaldehyde (4); (a) Dibenzoyl peroxide, NBS, CCl₄, reflux, 24 h; (b) 4-methylpiperazine, CHCl₃, 24 h; (c) Raney Nickel alloy, formic acid 75%, reflux, 2 h.

Table1. Chemical structure of synthesized 3-benzylidene indole-2-ones (IV_{a-c}) and 3-phenyliminoindole-2-ones (V_{a-h}).

ID	R ₁	R ₂	R ₃
IV _a	CN		Br
IV _b	NHCO ₂ H ₃	F	
IV _c	NHCOCH ₃	Cl	
IV _d			
IV _e			

ID	R ₄	R ₅	R ₆
V _a	CH ₃	F	
V _b	OH	F	
V _c	CONH ₂	F	
V _d	Cl	F ₃ CO	
V _e	CH ₃	F ₃ CO	
V _f	OH	F ₃ CO	
V _g	COH ₂	F ₃ CO	
V _h	CONH ₂	Cl	Cl

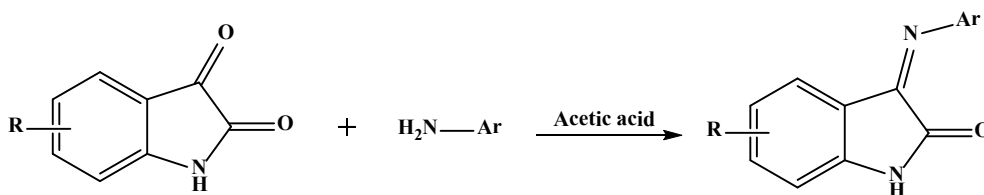
with an Agilent 6400 Series equipped with an electrospray ionization source (capillary voltage at 4000V, nebulizing gas temperature at 300 °C, nebulizing gas flow at 12 L/ min) . All the compounds were analyzed for C, H, N, and S on a Costech model 4010 and agreed with the proposed structures within $\pm 0.4\%$ of the theoretical values.

General procedure for synthesis of 3-(Substituted benzylidenyl)-indolin-2-one analogues (Compounds IV_{a-e})

A reaction mixture of the proper oxindole (1 equiv), aldehyde (1.2 equiv), and piperidine (0.1 equiv) in ethanol (1-2 mL/1 μ mol oxindole) was stirred at 90 °C for 3-5 h (15). After the mixture cooled, the precipitate was filtered, washed with cold ethanol and hexane and recrystallized from suitable solvent to give the target compound.

4-((5-bromo-2-oxoindolin-3-ylidene)methyl) benzonitrile (IV_d)

Yield 58%, mp 267-272.5 °C (dec.); ethanol; IR (KBr) ν_{\max} 3195 (NH), 2246 (nitrile), 1712 (C=O), 1613 cm^{-1} ; ¹H NMR (250 MHz, DMSO-*d*₆) δ 10.86 (s, 1H, NH), 8.4 (d, 2H, *J* = 8.25; H-3', 5'), 7.9 (m), 7.71 (s, 1H, H-vinyl), 7.4 (m), 6.83 (dd, 2H, *J* = 15, 8.25; H-2', 6'), mixture of *Z* and *E* isomers; ¹³C-NMR (62.9 MHz, DMSO-*d*₆) δ 116.2, 116.7, 116.9, 117.5, 117.9, 123.2, 123.4, 127.1, 127.9, 129.5, 131.2, 133.1, 133.3, 134.6, 136.6, 136.8, 137.3, 137.8, 140, 140.7, 142.6, 143.6, 145, 147.1, 171.1, 172.4; ESI-MS: Observed ($M+H^+$) = 325, 327 ($M+Na^+$) = 347, 349. Calcd for C₁₆H₉BrN₂O = 325.16; Anal. Found: C, 59.21; H, 2.78; Br, 24.59; N, 8.60; O, 4.91. Calcd for C₁₆H₉BrN₂O: C, 59.10; H, 2.79; Br, 24.57; N, 8.62; O, 4.92%.

Figure 5. Compounds V_{a-h} synthesis scheme.

N-(2-fluoro-4-((2-oxoin dolin-3-ylidene)methyl)phenyl)acetamide (IV_b)

Yield 15%, mp 249-252 °C (dec.); ethanol; IR (KBr) ν_{\max} 3185 (NH), 3175 (NH of acetamide), 1710 (C=O), 1660 (C=O of acetamide), 1613 cm^{-1} ; ^1H NMR (250 MHz, DMSO-*d*₆) δ 10.69 (s, 1H, NH-1), 10 (s, 1H, NH of acetamide), 8.14 (dt, 1H, *J*= 8.5, 1.75; H-6), 7.72(m), 7.23(dd, 2H, *J*=15,7.5; H-5', 6'), 6.9 (m), 2.14 (s, 3H, NHCOCH₃-4'), mixture of *Z* and *E* isomers; ^{13}C -NMR (62.9 MHz, DMSO-*d*₆) δ 109.3, 110.1, 116.2, 116.5, 117.5, 117.8, 118.6, 119.6, 120.6, 121.1, 121.2, 122.3, 123, 124, 125.6, 126.3, 127.4, 127.5, 127.7, 128.9, 129.5, 129.7, 130.2, 130.4, 130.5, 130.7, 134.2, 140.6, 142.9, 150.5, 154.4, 167.2, 168.5, 169, 169.1; Anal. Found: C, 68.93; H, 4.41; F, 6.43; N, 9.47; O, 10.82. Calcd for C₁₇H₁₃FN₂O₂: C, 68.91; H, 4.42; F, 6.41; N, 9.45; O, 10.80%.

N-(2-chloro-4-((2-oxoin dolin-3-ylidene)methyl)phenyl) acetamide (IV_c)

Yield 56%, mp 220-228 °C (dec.); ethanol; IR (KBr) ν_{\max} 3184 (N-H), 3082 (NH of acetamide), 1702 (C=O), 1662 (C=O of acetamide), 1611 cm^{-1} ; ^1H NMR (250 MHz, DMSO-*d*₆) δ 10.65 (s, 1H, NH-1), 9.66 (s, 1H, NH of acetamide), 7 (m), 2.15 (s, 3H, NHCOCH₃-4'), mixture of *Z* and *E* isomers; ^{13}C -NMR (62.9 MHz, DMSO-*d*₆) δ 23.5, 23.6, 109.4, 110.2, 119.8, 120.6, 121.1, 121.2, 122.2, 124, 124.4, 124.6, 125, 125.4, 126.8, 127.8, 128.2, 129.1, 130.2, 130.3, 131.2, 131.5, 131.7, 132.1, 133.8, 134.6, 136, 136.5, 140.7, 142.9, 167.1, 168.5, 169; Anal. Found: C, 65.31; H, 4.2; Cl, 11.36; N, 8.95; O, 10.21. Calcd for C₁₇H₁₃ClN₂O₂: C, 65.29; H, 4.19; Cl, 11.34; N, 8.96; O, 10.23%

Preparation of 4-(bromomethyl)benzonitrile(2) 4-toluenitrile (1) (0.1 mol) was added to a

flask containing N-bromosuccinimide (0.11 mol) and dibenzoyl peroxide (500 mg) in dried carbon tetrachloride (200 mL). The reaction mixture was refluxed under nitrogen atmosphere overnight. Then the mixture cooled and filtered and the filtrate was concentrated and 300 mL hexane was added to this solution to form the white crystals of 4-(bromomethyl) benzonitrile (16). The product was purified by recrystallization from chloroform. The yield was (50%), mp = 115-117 °C.

Preparation of 4-((4-methylpiperazin-1-yl)methyl)benzonitrile(3)

1-(Bromo) toluenitrile (10.2mmol) in 20 mL of chloroform was stirred at room temperature before dropwise addition of a solution of 1-methyl piperazine (28 mmol) in 5 mL chloroform. The reaction mixture was stirred at room temperature for 24 h then the reaction was quenched with water and further stirred for 30 min before extracting with chloroform. The organic layer was dried and concentrated (17). In the residue were formed crystals which were washed with hexane. It was pure 4-((4-methylpiperazin-1-yl)methyl) benzonitrile; yield (35%), mp = 65-67°C (Ref: 62-64°C); ESI-MS: Observed (M+H⁺) = 216. Calcd for C₁₃H₁₇N₃ = 215.2.

Preparation of 4-((4-methylpiperazin-1-yl)methyl)benzaldehyde (4) (18)

4-((4-methylpiperazin-1-yl)methyl) benzonitrile (9 mmol) was dissolved in formic acid 75% (37 mL) and raney nickel alloy (2 g) was added to this solution. The mixture was refluxed for 2 h then filtered with celite and washed with 20 mL of cold ethanol 96°. The filtrate was concentrated and again filtered to remove the green colloidal impurities to give (1.8 g) crude product, ESI-MS: Observed (M+H⁺) =

219. Calcd for $C_{13}H_{18}N_2O = 218.29$

Synthesis of 3-(4-((4-methylpiperazin-1-yl)methyl)benzylidene)indolin-2-one (IV_d)

A reaction mixture of oxindole (1 equiv), 4-((4-methylpiperazin-1-yl)methyl)benzaldehyde (1.2 equiv), and piperidine (0.1 equiv) in ethanol (1-2 mL / 1 μ mol oxindole) was stirred at 90 °C overnight. The solvent of the reaction mixture was evaporated and the residue was dissolved in warm ethyl acetate and passed through a column of silica gel. The polarity of eluting solvent was increased with the addition of methanol to the ethyl acetate. The yellow liquid phase was collected and the solvent was evaporated to achieve 3-(4-((4-methylpiperazin-1-yl)methyl)benzylidene)indolin-2-one: Yield 38%, mp 264-269 °C (dec.); ethanol; ¹H NMR (500 MHz, DMSO-*d*₆) δ 10.58 (s, 1H, NH-1), 7.66 (d, 2H, *J* = 8, H-2',6'), 7.59 (s, 1H, H-vinyl), 7.56 (d, *J* = 8, 1H, H-4), 7.43 (d, 2H, *J* = 8, H-3',5'), 7.21 (t, 1H, *J* = 7.5, H-6), 6.85 (m, 2H, H-5,7), 3.52 (s, 2H, CH₂), 2.37 (m, 8H, CH₂ piperazine), 2.16 (s, 3H, CH₃); ¹³C-NMR (62.9 MHz, DMSO-*d*₆) δ 45.2, 52.1, 54.3, 61.5, 110.1, 120.8, 121.1, 122.3, 127.1, 128.5, 129, 129.2, 130, 131.8, 132.9, 135.7, 140.2, 142.8, 164.6, 168.6; ESI-MS: Observed (M+H⁺) = 334. Calcd for C₂₁H₂₃N₃O = 333.43; Anal. Found: C, 75.70; H, 6.93; N, 12.58; O, 4.78. Calcd for C₂₁H₂₃N₃O: C, 75.65; H, 6.95; N, 12.60; O, 4.80%.

3-((5-(4-fluorophenyl)pyridin-3-yl)methylene)indolin-2-one (IV_e)

Yield 85%, mp 204-206.9 °C (dec.); ethanol; IR (KBr) ν_{\max} 3160 (N-H), 1720 (C=O), 1689, 1607 cm⁻¹; ¹H NMR (250 MHz, DMSO-*d*₆) δ 10.70 (s, 1H, NH), 9.23 (t, 1H, *J* = 2; H-2'), 9.15 (d, 1H, *J* = 1.75; H-6'), 8.9 (m, 1H, H-4'), 7.91 (s, 1H, H-vinyl), 7.84 (m), 7.72 (t, 1H, *J* = 7.5 Hz; H-4), 7.39 (m), 7.26 (t, 1H, *J* = 7.5; H-5), 7.05 (t, 1H, *J* = 7.5; H-6), 6.87 (m, 1H; H-7), mixture of Z and E isomers; ¹³C-NMR (62.9 MHz, DMSO-*d*₆) δ 115.9, 116.2, 120.2, 120.7, 121.3, 122.1, 124.3, 128.9, 129, 129.1, 129.6, 129.8, 130.6, 130.7, 131.8, 132.4, 132.7, 132.8, 133.3, 133.7, 134.3, 135.6, 141.1, 143.2, 147.9, 148, 148.1, 150.9, 160.5, 164.4, 167, 168.1; ESI-MS: Observed (M+H⁺) = 317. Calcd for C₂₀H₁₃FN₂O = 316.3.

Anal. Found: C, 75.96; H, 4.15; F, 6.03; N, 8.84; O, 5.05. Calcd for C₂₀H₁₃FN₂O: C, 75.94; H, 4.14; F, 6.01; N, 8.86; O, 5.06%.

General Procedure for Compounds (V_{a-h})

A mixture of indole-2, 3-dione (0.01 M) and Amine (0.01 M) in absolute ethanol (20 mL) was refluxed for 20 h in the presence of 2-3 drops of glacial acetic acid (19). After cooling, the resultant was filtered and washed with hexane and recrystallised from appropriate solvent to give compounds V_{a-h}.

5-fluoro-3-(p-tolylimino)indolin-2-one (V_a)

Yield 38%, mp 264-269 °C (dec.); ethanol; IR (KBr) ν_{\max} 3256 (N-H), 1741 (C=O), 1652 (C=N), 1613 cm⁻¹; ¹H NMR (250 MHz, DMSO-*d*₆) δ 11.03 (s, 1H, NH-1), 7.15 (m, 6H, H-6,7,2',3',5',6'), 6.14 (dd, 1H, *J* = 8.5, 2.5 Hz; H-4), 2.37 (s, 3H, CH₃-4'), mixture of Z and E isomers; ¹³C-NMR (62.9 MHz, DMSO-*d*₆) δ 20.5, 111.5, 111.9, 112.5, 112.6, 115.9, 116, 117.4, 119.8, 120.5, 120.9, 128.7, 130, 134.6, 143.2, 147.2, 154.3, 154.8, 158.5, 163.5; ESI-MS: Observed (M+H⁺) = 255. Calcd for C₁₅H₁₁FN₂O = 254.2; Anal. Found: C, 70.89; H, 4.31; F, 7.45; N, 11.03; O, 6.27. Calcd for C₁₅H₁₁FN₂O: C, 70.86; H, 4.36; F, 7.47; N, 11.02; O, 6.29%.

5-fluoro-3-(4-hydroxyphenylimino)indolin-2-one (V_b)

Yield 74%, mp 307-320 °C (dec.); ethanol; IR (KBr) ν_{\max} 3298, 1711 (C=O), 1619, 1599 (C=N) cm⁻¹; ¹H NMR (250 MHz, DMSO-*d*₆) δ 10.97 (s, 1H, NH-1), 9.66 (s, 1H, OH-4'), 7.26 (m), 6.84 (m), 6.44 (dd, 1H, *J* = 8.5, 2.5 Hz; H-4), mixture of Z and E isomers; ¹³C-NMR (62.9 MHz, DMSO-*d*₆) δ 108.6, 109, 111.1, 111.3, 111.5, 112.3, 112.4, 114.7, 115.9, 116, 116.2, 119, 119.4, 119.9, 120.1, 120.5, 124.6, 138.8, 140.7, 141, 142.9, 143, 153.4, 154.8, 155.8, 156.9, 158.6, 158.9, 163.7; ESI-MS: Observed (M+H⁺) = 257. Calcd for C₁₄H₉FN₂O₂ = 256.23; Anal. Found: C, 65.68; H, 3.53; F, 7.42; N, 10.91; O, 12.47. Calcd for C₁₄H₉FN₂O₂: C, 65.62; H, 3.54; F, 7.41; N, 10.93; O, 12.49%.

4-(5-fluoro-2-oxindolin-3-ylideneamino)benzamide (V_c)

Yield 19%, mp 277-310 °C (dec.); ethanol;

IR (KBr) ν_{\max} 3394 (NH₂), 3232 (N-H), 1741 (C=O Oxindole), 1673 (C=O benzamide), 1630 (C=N) cm⁻¹; ¹H NMR (250 MHz, DMSO-*d*₆) δ 11.09 (s, 1H, NH-1), 7.94(m), 7.33 (m), 7.06 (m), 6.92 (m), 5.97(dd, 1H, *J* = 8.25, 2.5; H-4), mixture of *Z* and *E* isomers; ¹³C-NMR (62.9 MHz, DMSO-*d*₆) δ 109.7, 110.1, 111.7, 112.1, 112.7, 112.8, 115.8, 115.9, 116.9, 118.2, 120.9, 121.3, 127.9, 129.2, 130.8, 143.4, 152.4, 163.3, 167.3, 167.6; ESI-MS: Observed (M+H⁺) = 284. Calcd for C₁₅H₁₀FN₃O₂ = 283.2; Anal. Found: C, 63.70; H, 3.55; F, 6.72; N, 14.85; O, 11.33. Calcd for C₁₅H₁₀FN₃O₂: C, 63.60; H, 3.56; F, 6.71; N, 14.83; O, 11.30%.

3-(4-chlorophenylimino)-5-(trifluoromethoxy)indolin-2-one (V_d)

Yield 43%, mp 230-240 °C (dec.); ethanol; IR (KBr) ν_{\max} 3282 (N-H), 1746 (C=O), 1628 (C=N) cm⁻¹; ¹H NMR (250 MHz, DMSO-*d*₆) δ 11.21 (s, 1H, NH-1), 7.46 (m), 7.06 (m), 6.20 (d, 1H, *J* = 1.25 Hz; H-4), mixture of *Z* and *E* isomers; ¹³C-NMR (62.9 MHz, DMSO-*d*₆) δ 112, 112.7, 116, 117.8, 119.3, 121.1, 127.3, 127.4, 128.2, 129.4, 129.5, 142.1, 143.3, 144.7, 146, 147.3, 148.7, 152.9, 154.8, 158.4, 163.3. 6; ESI-MS: Observed (M+H⁺) = 342. Calcd for C₁₅H₈ClF₃N₂O₂ = 340.7; Anal. Found C, 52.92; H, 2.36; Cl, 10.43; F, 16.75; N, 8.21; O, 9.37. Calcd for C₁₅H₈ClF₃N₂O₂: C, 52.88; H, 2.37; Cl, 10.41; F, 16.73; N, 8.22; O, 9.39%.

3-(p-tolylimino)-5-(trifluoromethoxy)indolin-2-one (V_e)

Yield 59%, mp 266.5-268.5 °C (with dec.); ethanol; IR (KBr) ν_{\max} 3254 (N-H), 1741 (C=O), 1619(C=N) cm⁻¹; ¹H NMR (250 MHz, DMSO-*d*₆) δ 11.3 (s, 1H, NH-1), 7.15(m, 6H, H- 6, 7, 2', 3', 5', 6'), 6.25 (d, 1H, *J* = 1.25 Hz, H-4), 2.36 (s, 3H, CH₃-4'), mixture of *Z* and *E* isomers; ¹³C-NMR (62.9 MHz, DMSO-*d*₆) δ 20.41, 20.55, 111.8, 112.6, 116.1, 117.3, 117.8, 119.9, 127.1, 128.7, 129.9, 134.7, 142.1, 145.7, 147.4, 154.1, 163.4; ESI-MS: Observed (M+H⁺) = 321. Calcd for C₁₆H₁₁F₃N₂O₂ = 320.2; Anal. Found: C, 60.2; H, 3.45; F, 17.81; N, 8.74; O, 9.97. Calcd for C₁₆H₁₁F₃N₂O₂: C, 60.00; H, 3.46; F, 17.80; N, 8.75; O, 9.99%.

3-(4-hydroxyphenylimino)-5-(trifluoromethoxy)indolin-2-one (V_f)

Yield 80%, mp 230-238 °C (dec.); ethanol; IR (KBr) ν_{\max} 3314 (O-H), 3209 (N-H), 1732 (C=O), 1627 (C=N) cm⁻¹; ¹H NMR (250 MHz, DMSO-*d*₆) δ 11.13 (1H, s, NH-1), 9.66 (s, 1H, OH-4'), 7.37 (m), 6.89(m), 6.60 (d, 1H, *J* = 1.25 Hz; H-4), mixture of *Z* and *E* isomers; ¹³C-NMR (62.9 MHz, DMSO-*d*₆) δ 111.5, 112.4, 114.7, 115.9, 116.3, 117.5, 119.9, 123.8, 124.8, 125.8, 126.8, 138.7, 141, 142.1, 143.4, 145.6, 149.1, 153.2, 155.9, 157.1, 158.9, 163.7; ESI-MS: Observed (M+H⁺) = 323. Calcd for C₁₅H₉F₃N₂O₃ = 322.2; Anal. Found: C, 55.93; H, 2.84; F, 17.67; N, 8.69; O, 14.92. Calcd for C₁₅H₉F₃N₂O₃: C, 55.91; H, 2.82; F, 17.69; N, 8.69; O, 14.90%.

4-(2-oxo-5-(trifluoromethoxy)indolin-3-ylideneamino)benzamide (V_g)

Yield 49%, mp 296-304 °C (dec.); ethanol; IR (KBr) ν_{\max} 3429 (NH₂), 3121 (N-H), 1727 (C=O Oxindole), 1697 (C=O benzamide), 1608 (C=N) cm⁻¹; ¹H NMR (250 MHz, DMSO-*d*₆) δ 11.22 (brs, 3H, NH-1, CONH₂-4'), 7.48 (m, 6H, H-6, 7, 2', 3', 5', 6'), 6.10 (s, 1H, H-4), mixture of *Z* and *E* isomers; ¹³C-NMR (62.9 MHz, DMSO-*d*₆) δ 112, 112.7, 116, 116.8, 118, 118.3, 121.8, 122.1, 127.4, 127.9, 129.1, 130.1, 130.8, 142.1, 143.3, 144.8, 145.9, 151.5, 152.5, 154.4, 158.4, 163.2, 167.1, 167.6; ESI-MS: Observed (M+H⁺) = 350. Calcd for C₁₆H₁₀F₃N₃O₃ = 349.26; Anal. Found: C, 55.05; H, 2.88; F, 16.30; N, 12.05; O, 13.72. Calcd for C₁₆H₁₀F₃N₃O₃: C, 55.02; H, 2.89; F, 16.32; N, 12.03; O, 13.74%.

4-(5, 7-dichloro-2-oxoindolin-3-ylideneamino)benzamide (V_h)

Yield 58%, mp 322-325 °C (dec.); ethanol; IR (KBr) ν_{\max} 3460 (NH₂), 3339 (N-H), 1733(C=O Oxindole), 1662(C=O benzamide), 1610(C=N) cm⁻¹; ¹H NMR (250 MHz, DMSO-*d*₆) δ 11.65 (s, 1H, NH-1), 11.51 (s, 2H, CONH₂-4'), 7.3 (m), 6.18 (d, 1H, *J* = 1.75 Hz; H-4), mixture of *Z* and *E* isomers; ¹³C-NMR (62.9 MHz, DMSO-*d*₆) δ 112.4, 115.8, 116.6, 116.9, 117.9, 118.4, 120.6, 120.8, 121.3, 122.8, 123.3, 123.9, 125.6, 126.8, 127, 127.9, 129, 129.2, 130.3, 131, 133, 135, 142.2, 143.6, 146.5, 151.2, 151.6, 152,

Table 2. IC₅₀ values of Compound IV_{a-e}, V_{a-h} and Tamoxifen on MCF7 and HT29 cell lines by MTT assay.

Comp. ID.	MCF-7 ^a		HT29 ^b	
	IC ₅₀ (μ M)	RSD%	IC ₅₀ (μ M)	RSD%
IV _a	6	7.9	13.13	4.3
IV _b	9.3	5.0	10.75	6.8
IV _c	>100	-	38.27	4.3
IV _d	42.07	3.8	7.51	6.9
IV _e	8.54	6.7	>100	-
V _a	>100	-	>100	-
V _b	>100	-	>100	-
V _c	>100	-	>100	-
V _d	>100	-	90.31	2.1
V _e	>100	-	>100	-
V _f	>100	-	>100	-
V _g	27.2	7.1	61.9	3.4
V _h	65.86	8.1	>100	-
TAMOXIFEN	6.05	4.9	10.36	3.7

^a human breast adenocarcinoma cell line. ^b human colon adenocarcinoma cell line

153.4, 158.1, 159.4, 163, 167.2, 167.5, 168; ESI-MS: Observed (M+H⁺) = 335. Calcd for C₁₅H₉Cl₂N₃O₂ = 334; Anal. Found: C, 53.91; H, 2.70; Cl, 21.24; N, 12.58; O, 9.59. Calcd for C₁₅H₉Cl₂N₃O₂: C, 53.91; H, 2.71; Cl, 21.22; N, 12.57; O, 9.58%.

Cytotoxic activity

Cell lines

HT-29 (human colon adenocarcinoma cell line) and MCF-7 cells (human breast adenocarcinoma cell line) were used for the *in-vitro* screening of newly synthesized compounds. Cell lines were obtained from Pasteur Institute of Iran, Tehran, Iran. Each cell line was cultured in suitable medium for desired growth, plus 10% FBS and 1% penicillin-streptomycin in a humidified incubator at 37°C in an atmosphere of 95% O₂ and 5% CO₂. Then the growth curve of each cell line was plotted.

Subculture of adherent cell lines

Cultures were observed using an inverted microscope to assess the degree of confluency and the absence of bacterial and fungal contaminants was confirmed. Cell monolayer was washed with PBS (Phosphate buffer solution) using a

volume equivalent to half the volume of culture medium. Trypsin was added on to the washed cell monolayer, flask was rotated to cover monolayer with trypsin. Flask was returned to the incubator and left for 2-10 min. The cells were examined using an inverted microscope to ensure that all the cells were detached and floated. The cells were resuspended in a small volume of fresh serum containing appropriate medium. A 100 μ L aliquot was removed to perform a cell count. The required number of cells were transferred to plates (tissue culture grade, 96 wells, round bottom) and incubated for 24 h at 37 °C and 5 % CO₂.

MTT assay

Cell viability was assessed by MTT assay (microculture tetrazolium/formazan assay). After 24 h the medium of the cells which were seeded in 96 well plates was replaced by fresh medium containing compounds, each experiment was done in triplicate and six concentrations (20, 21). Tamoxifen was used as positive control. Then, the cells were incubated at 37°C for 72 h, the medium was changed by fresh medium containing MTT ([3-(4, 5-dimethylthiazol-2-yl)-2,4-diphenyltetrazolium bromide]) with a final concentration of 0.5 mg/

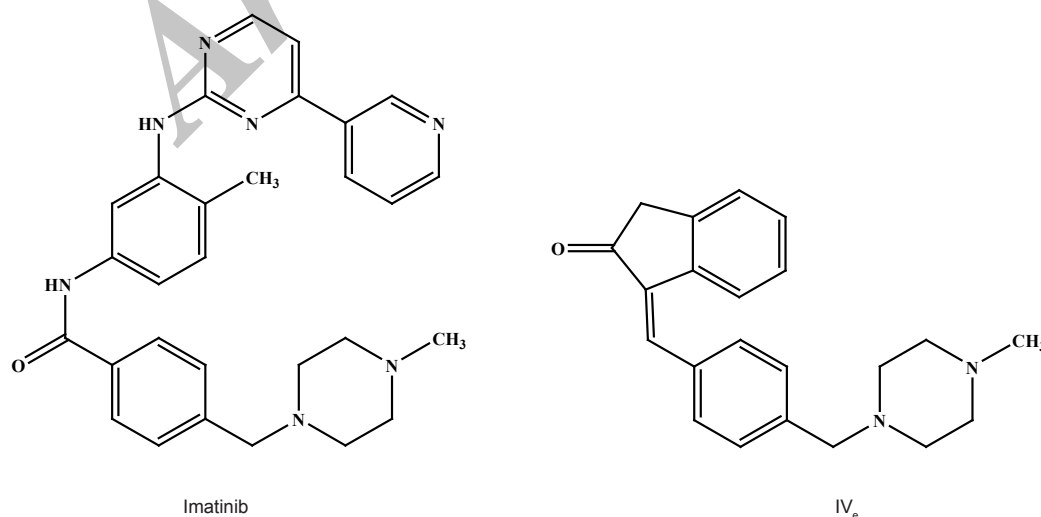
Table 3. Global physicochemical properties for compounds IV_{a-c} and V_{a-h}.

Compound	CLogP ^a	P ^b	V ^c	SA ^d	DM ^e
IV _a	3.7836	1057.02	81.58	330.762	4.144
IV _b	3.7808	5454.61	92.26	123.949	2.146
IV _c	2.0496	2139.00	81.36	300.439	4.293
IV _d	2.3696	3488.20	85.56	118.548	3.918
IV _e	3.538	5205.99	103.14	192.032	3.159
V _a	3.292	926.57	71.75	571.2	1.158
V _b	2.126	1049.3	67.67	133.9	2.338
V _c	1.306	1166.7	75.06	249.2	5.4
V _d	4.391	1648.15	78.9	235.2	2.44
V _e	4.177	1725.83	80.2	203.6	2.044
V _f	3.011	1747.35	76.11	216.02	2.957
V _g	2.191	1456.59	83.51	128.59	5.53
V _h	2.589	1233.72	83.87	451.84	4.027

^a ClogPs were calculated by using ChemDraw Ultra version 8.0. ^b Polarizability (\AA^3) values were calculated by using ChemDraw Ultra version 8.0. ^c Molecular volume (\AA^3) values were calculated by using ChemDraw Ultra version 8.0. ^d Surface area (\AA^2) values were calculated by using ChemDraw Ultra version 8.0. ^e Dipole moment (debye) values were calculated by using ChemDraw Ultra version 8.0.

mL. The cells were incubated for another 4 h in a humidified atmosphere at 37°C and after that the medium containing MTT was removed and remaining MTT formazan crystals were dissolved in 200 μL DMSO. The absorbance was measured at 570 nm after 20 min shaking using an ELISA reader. IC₅₀ was defined as the concentration of the compounds that produced a 50% decrease in cell viability relative to the

negative control which was wells exposed to the solvent without any compound. IC₅₀ was determined by plotting a graph of Log (concentration of compound) vs % effect (cell viability). A line drawn from 50 % value on the Y axis meets the curve and interpolate to the X axis. The X axis value gives the Log (concentration of compound). The antilog of that value gives the IC₅₀ value.

**Figure 6.** chemical structures of Imatinib and compound IV_e.

Results and Discussion

All the synthesized compounds were assayed for their cytotoxic activities against colon cancer cell lines (HT29) and breast cancer cell lines (MCF7). The results are presented in Table 2.

As it appears from Table 2, the most active compounds are among the 3-benzylidene indole-2-one derivatives (IV series). In 3-phenyliminoindole-2-ones (V series) only compound V_g showed moderate cytotoxic activity (27.2 μ M against MCF7 and 61.9 μ M against HT29). Compounds IV_a and IV_b in 3-benzylidene indole-2-one series showed the best activities against both MCF7 and HT29 cell lines. The most potent compound against MCF7 breast cancer cell is compound IV_a which bears 5-bromo substitution. This is in complete agreement with the report by Olgen *et al.* for a series of N-benzyl-indole derivatives. They have found the 5-bromo substituted derivatives as the most potent inhibitors of c-Src tyrosine kinase.

Compound IV_d which shows highest activity against HT29 (7.51 μ M) has a moderate activity (42.07 μ M) against MCF7.

Compound IV_d is a 3-benzylidene indole-2-one derivative with a methyl piperazine moiety similar to the structure of Imatinib which is a 2-phenylaminopyrimidine derivative that functions as a specific inhibitor of a number of tyrosine kinase enzymes (Figure 6).

Global physicochemical properties for compounds IV_{a-c} and V_{a-h} were calculated using Chemdraw Ultra version 8.0 and the results are presented in Table 3.

Efforts to find a relationship between these physicochemical parameters and cytotoxic activities of the compounds did not result in a clear correlation. The only interesting point in this regard is the closeness of CLogP values for the two most active compounds IV_a and IV_b which are 3.7836 and 3.7808 respectively.

Studies are in progress to examine whether further structural modifications, specially with regard to substituents at the 5 position, can result in enhancement of the cytotoxic activity of 3-benzylidene indole-2-one derivatives. Since MTT assay is a general screening method to evaluate the cytotoxicity of compounds and lacks

specificity for anti-cancer evaluation, further mechanistic studies are needed to demonstrate the anti cancer and anti apoptosis activity of these compounds.

Src family of tyrosine kinases and other members of kinase enzymes which are proven to be involved in human colon and/or breast cancer are among the best candidates for the future enzyme inhibition studies.

Acknowledgements

The authors would like to express their thanks to the research deputy of Shahid Beheshti University of Medical Sciences for financial support of this research.

References

- (1) Chen I, Safe S and Bjeldanes L. Indole-3-carbinol and diindolylmethane as aryl hydrocarbon (Ah) receptor agonists and antagonists in T47D human breast cancer cells. *Biochem. Pharmacol.* (1996) 51: 1069-1076.
- (2) Suzen S and Buyukbingol E. Anti-cancer activity studies of indolalithiohydantoin (PIT) on certain cancer cell lines. *Il Farmaco* (2000) 55: 246-248.
- (3) Giagoudakis G and Markantonis SL. Relationships between the concentrations of prostaglandins and the nonsteroidal antiinflammatory drugs indomethacin, diclofenac, and ibuprofen. *Pharmacotherapy* (2005) 25: 18-25.
- (4) Buyukbingol E and Suzen S. Studies on the synthesis and structure-activity relationships of 5-(3'-indolyl)-2-thiohydantoin derivatives as aldose reductase enzyme inhibitors. *Il Farmaco* (1994) 49: 443-447.
- (5) Suzen S and Buyukbingol E. Evaluation of anti-HIV activity of 5-(2-phenyl-3'-Indolyl)-2-thiohydantoin. *Il Farmaco* (1998) 53: 525-527.
- (6) Walter G, Liebl R and von Angerer E. 2-phenylindole sulfamates: inhibitors of steroid sulfatase with antiproliferative activity in MCF-7 breast cancer cells. *J. Steroid. Biochem. Mol. Biol.* (2004) 88: 409-420.
- (7) Biberger C and von Angerer E. Phenylindoles with sulfur containing side chains. Estrogen receptor affinity, antiestrogenic potency, and antitumor activity. *J. Steroid. Biochem. Mol. Biol.* (1996) 58: 31-43.
- (8) Ge X, Yannai S, Rennert G, Gruener N and Fares FA. 3, 3' -diindolylmethane induces apoptosis in human cancer cells. *Biochem. Biophys. Res. Commun.* (1996) 228: 153-158.
- (9) Weng J, Tsai C, Kulp S and Chen C. Indole-3-carbinol as a chemopreventive and anti-cancer agent. *Cancer Lett.* (2008) 262: 153-163.
- (10) Aggarwal BB and Ichikawa H. Molecular targets and anticancer potential of indole-3-carbinol and its derivatives. *Cell Cycle* (2005) 4: 1201-1215.

- (11) Olgen S, Akaho E and Nebioglu D. Evaluations of indole esters as inhibitors of p60^{c-src} receptor tyrosine kinase and investigation of enzyme inhibition using receptor docking studies. *J. Enzyme Inhib. Med. Chem.* (2003) 18: 485-490.
- (12) Olgen S, Akaho E and Nebioglu D. Synthesis and docking-based studies of 3-substituted indolin-2-on and thione derivatives as tyrosine kinase inhibitors. *Il Farmaco* (2005) 60: 497-506.
- (13) Sirvent A, Bénistant C, Pannequin J, Veracini L, Simon V, Bourgaux JF, Hollande F, Cruzalegui F and Roche S. Src family tyrosine kinases-driven colon cancer cell invasion is induced by Csk membrane delocalization. *Oncogene* (2010) 29: 1303-15.
- (14) Biscardi JS, Ishizawar RC, Silva MC and Parsons SJ. Tyrosine kinase signalling in breast cancer: Epidermal growth factor receptor and c-Src interactions in breast cancer. *Breast Cancer Res.* (2000) 2: 203-210
- (15) Sun L, Tran N, Tang F, App H, Hirth P, McMahon G and Tang C. Synthesis and biological evaluations of 3-substituted indolin-2-ones: a novel class of tyrosine kinase inhibitors that exhibit selectivity toward particular receptor tyrosine kinases. *J. Med. Chem.* (1998) 41: 2588- 2603.
- (16) Schlenoff JB, Johnson KF, Dharia J and Gao F. (Florida State University) US Patents 005552551A (1996).
- (17) Sairam P, Puranik R, Kelkar AS, Sasikiran S, Veerender M and Parvathi A. The ester and amide derivatives of 4-(4-methyl piperazinomethyl) benzoic acid. *Synth. Commun.* (2003) 33: 3597-3605.
- (18) van Es T and Staskun B. Aldehydes from aromatic nitriles: 4- formylbenzenesulfonamide. *Org. Synth. Coll. Vol.* (1988) 6: 631.
- (19) Ravichandran V, Mohan S and Kumar KS. Synthesis and antimicrobial activity of Mannich bases of Isatin and its derivative with 2-[(2, 6- dichlorophenyl)amino] phenylacetic acid. *ARKIVOC* (2007) 14: 51-57.
- (20) Alley MC, Scudiero DA, Monkes A, Hursey ML, Czerwinski MJ, Fine DL, Abbott BJ, Mayo JG, Shoemaker RH and Boyd MR. Feasibility of drug screening with panel of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res.* (1988) 48: 589-601.
- (21) Sadeghi-aliabadi H, Emami A, Saidi M, Sadeghi B and Jafarian A. Evaluation of in-vitro cytotoxic effects of *Juniperus foetidissima* and *Juniperus sabina* extracts against a panel of cancer cells. *Iranian J. Pharm. Res.* (2009) 8: 281-286.

This article is available online at <http://www.ijpr.ir>