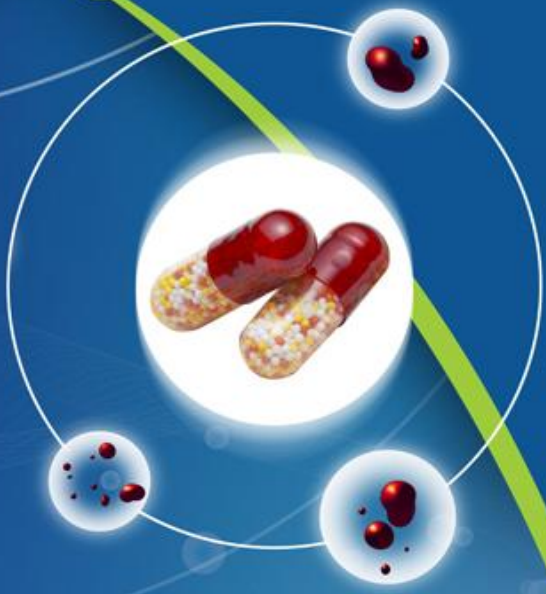
ISSN : 2320 4850

BI MONTHLY

Asian Journal of Pharmaceutical Research And Development

(An International Peer Reviewed Journal of Pharmaceutical Research and Development)



AJPRD

Volume - 02 Issue - 02 MAR-APR 2014

website: www.ajprd.com

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Asian Journal of Pharmaceutical Research and Development
(An International Peer-Reviewed Journal of Pharmaceutical Research and Development)
www.ajprd.com
ISSN 2320-4850

Research Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL INDOLE DERIVATIVES FOR ANTICANCER AND ANTITUBERCULAR ACTIVTY

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Received: May 2014 ***Revised and Accepted: June 2014***

ABSTRACT

A series of substituted indole derivatives were synthesized by condensation of 2-(o-Aminophenyl)indole with various benzaldehydes to form N-(Arylidine)-2-(1H indol-2-yl)-benzenamines ***(2a-2l)*** *which further react with isatin and ammonium acetate to give 3-[2-(1H-Indol-2-yl)phenyl]-2aryl-imidazo[4,5-b]indoles* ***(3a-3l)****. The synthesized compounds were characterized by FTIR, ^{1}HNMR, mass spectra and elemental analysis. At last, all end products were evaluated for the antitubercular and anticancer activities.*

KEYWORDS:: *Indole, Antitubercular activity, Anticancer activity*

INTRODUCTION

Heterocyclic moieties are a part of most of the drugs existing in the market in one form or the other. Indole is one such moiety which is a part of various anti-inflammatory and analgesic drugs, as it played a crucial role in the development of the COX-2 inhibitors such as Indomethacin, Fendosal, Tenidap etc which occupied a good status in the market for a long time. The indole ring system is a valuable structural moiety having various biologically activities such as, cardioprotective [1], antiviral [2], antibacterial [3], antitumour [4], plant growth regulator [5], antioxidant [6], anti-inflammatory [7], analgesic [8] etc. So looking at the history of Indole and its use, it was thought worthwhile to synthesize some Indole derivatives and evaluate their antitubercular and anticancer activity.

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In this present work, Phenyl hydrazine on reaction with 2-Aminoacetophenone gave 2-(*o*-Aminophenyl)indole which on further reaction with various benzaldehyde derivatives gave N-(arylidine)-2-(1*H*-indol-2-yl)-benzenamines **(2a-2l)** then these derivatives were reacted with ammonium acetate and isatin to form 3-(2-(1*H*-indol-2-yl) phenyl)-2-arylimidazo[4,5-*b*] indoles **(3a-3l)**.

MATERIALS AND METHODS:

The purity of all the newly synthesized compounds were checked by TLC on silica gel-protected aluminum sheets (Type 60 F_{254}, Merck) and the spots were detected by exposure to iodine vapors and UV-lamp at λ 254 nm. The melting points were determined in open capillary tubes and were uncorrected. The infrared (FTIR) spectra were recorded on 470-Shimadzu infrared spectrophotometer using the KBr disc prepared by pressed pellet technique and expressed in cm^{-1}. ^{1}H NMR spectra were recorded on Bruker DRX-300 using DMSO-d_6 as a solvent. The chemical

shift was given in δ (ppm) in a downfield manner using tetramethylsilane (TMS) as an internal standard. Splitting patterns were designated as follows: s: singlet; d: doublet; m: multiplet. Elemental analysis was carried on Elemental Vario EL III Carlo Erba 1108 and the values were within ±0.04% of the theoretical values.

Synthetic Procedures

2-(*o*-Aminophenyl)indole (1)

Step I- Preparation of Phenyl Hydrazone

Phenyl Hydrazine (1.08 gm, 10 mmol) and 2-Aminoacetophenone (1.35 gm, 10 mmol) were mixed in ethanol (20 mL) with few drops of acetic acid and refluxed at 50-60 ^{o}C for 10 hours on a water bath. Crystals were filtered and washed with ethanol and then recrystallised with ethanol.

Step II- Cyclization of Phenyl Hydrazone

Methane sulfonic acid (10 mL) was heated to 80 ^{o}C and then 1.35 gm of P_2O_5 was added and stirred until it dissolved. Phenyl hydrazone (1 gm) was added slowly and mixture was heated between 80-100 ^{o}C for half an hour. Reaction mixture was poured on ice and neutralized with NaOH solution. Crude product was filtered and recrystallized with ethanol.

General Procedure

N-(arylidine)-2-(1*H*-indol-2-yl)-benzenamines (2a-2l)

To an equimolar mixture of 2-(*o*-Aminophenyl)indole & Ar-CHO, few drops of acetic acid were added with vigorous stirring. The resulting solution was heated under reflux for 3-4 hours. After cooling, the mixture was poured on ice and product was collected after filtration and recrystallised with ethanol.

3-(2-(1*H*-indol-2-yl) phenyl)-2-arylimidazo [4,5-*b*]indoles (3a-3l)

Equimolar mixture of intermediate **(2a-2l)**, isatin and ammonium acetate were mixed with ethanol and few drops of acetic acid were added. The mixture was refluxed for 10-14 hours. Then it was cooled to room temp.and poured over crushed ice. Crude product was collected and recrystallised with ethanol.

Derivatives synthesized:

3-(2-(1*H*-indol-2-yl) phenyl)-2-(*o*-hydroxyphenyl)imidazo[4,5-*b*]indole (3a):

IR (KBr, cm^{-1}) *v*: 3452.34(O-H, str.), 3338.55 (N-H, str.), 3041.53 (Ar C-H, str.), 1630.59 (Ar C=N, , str.), 1574.09 (C=C, str.), 1350.24 (Ar C-N, str.), 1283.29 (C-O str.). ^{1}H NMR (300 MHz, DMSO-d_6 in ppm): δ 5.099 (s, 1H, OH), 6.480(s, 1H, Ar-H), 6.711-6.811 (d, 2H, Ar-H), 7.005-7.360 (m, 6H, Ar-H), 7.319-7.414 (m, 4H, Ar-H), 7.429-7.624 (m, 4H, Ar-H) 10.029 (s, 2H, N-H, D_2O exchangable); MS (ESI) m/z: 440.4 (100) $[M]^+$, Elemental analysis: Anaylzed for $C_{29}H_{20}N_4O$: Found: C, 79.09%; H, 4.68%; N, 12.45%; O, 3.65%.

3-(2-(1*H*-indol-2-yl) phenyl)-2-(phenyl)imidazo[4,5-*b*]indole (3b):

IR (KBr, cm^{-1}) *v*: 3334.56 (N-H, str.), 3055.03 (Ar C-H, str.), 1602.74 (C=N, Aromatic, str.), 1563.45 (C=C, str.), 1365.51 (C-N, Aromatic, str.); ^{1}H NMR (300 MHz, DMSO-d_6 in ppm): δ 6.412(s, 1H, Ar-H), 7.000-7.329(m, 5H, Ar-H), 7.360-7.512 (m, 7H, Ar-H), 7.540-7.634 (m, 5H, Ar-H), 10.174 (s, 2H, N-H, D_2O exchangable); MS (ESI) m/z: 424.4 (100) $[M]^+$, Elemental analysis: Analyzed for $C_{29}H_{20}N_4$: Found: C, 82.02%; H, 4.77%; N, 13.25%.

3-(2-(1*H*-indol-2-yl) phenyl)-2-(2-nitrophenyl)imidazo[4,5-*b*]indole (3c):

IR (KBr, cm^{-1}) *v*: 3359.77 (N-H, str.), 3060.82 (Ar C-H, str.), 1614.31 (C=N, Aromatic, str.), 1582.31 (C=C, str.), 1525.64 (N=O, str.), 1323.77 (C-N, Aromatic, str.), 854.41 (C-N (Nitro)str.); ^{1}H NMR (300 MHz, DMSO-d_6 in ppm): δ 6.463(s, 1H, Ar-H), 7.003-7.080 (d, 3H, Ar-H), 7.191-7.406 (m, 6H, Ar-H), 7.451-7.782 (m, 6H, Ar-H), 8.254 (s, 1H, Ar-H), 10.010 (s, 2H, N-H, D_2O exchangable); MS (ESI) m/z: 469.7 (100) $[M]^+$, Elemental analysis: Analyzed for $C_{29}H_{19}N_5O_2$: Found: C, 74.16%; H, 4.06%; N, 14.88%; O, 6.85%.

3-(2-(1*H*-indol-2-yl) phenyl)-2-(3,4-dimethoxyphenyl)imidazo[4,5-*b*]indole (3d):

IR (KBr, cm^{-1}) *v*: 3346.96 (N-H, str.), 3056.96 (Ar.C-H, str.), 2929.67 (C-H, Aliphatic, str.), 1605.81 (C=N, str.), 1579.32 (C=C, str.), 1326.93 (C-N, str.), 1238.21 (C-O-C, str.); ^1H NMR (300 MHz, DMSO-d_6 in ppm): δ 3.731(s, 6H, $(OCH_3)_2$), 6.373 (s, 1H, Ar-H), 6.721-6.938 (m, 3H, Ar-H), 7.010-7.391 (m, 7H, Ar-H), 7.404-7.481 (d, 2H, Ar-H), 7.526-7.664 (d, 3H, Ar-H), 9.754 (s, 2H, N-H, D_2O exchangable); MS (ESI) m/z: 484.5 (100) $[M]^+$, Elemental analysis: Analyzed for $C_{31}H_{24}N_4O_2$: Found: C, 76.86%; H, 4.97; N, 11.59%; O, 6.57%.

3-(2-(1*H*-indol-2-yl) phenyl)-2-(3-nitrophenyl)imidazo[4,5-*b*]indole (3e):

IR (KBr, cm^{-1}) *v*: 3341.24 (N-H, str.), 3027.13 (Ar C-H, str.), 1605.39 (C=N, Aromatic, str.), 1560.45 (C=C, str.), 1510.50 (N=O, str.), 1312.63 (C-N, Aromatic, str.), 850.81 (C-N (Nitro)str.); ^1H NMR (300 MHz, DMSO-d_6 in ppm): δ 6.488(s, 1H, Ar-H), 7.004-7.224 (m, 5H, Ar-H), 7.263-7.428 (m, 5H, Ar-H), 7.450-7.835 (m, 5H, Ar-H),, 8.268 (s, 1H, Ar-H), 10.043 (s, 2H, N-H, D_2O exchangable); MS (ESI) m/z: 469.1 (100) $[M]^+$, Elemental analysis: Analyzed for $C_{29}H_{19}N_5O_2$: Found: C, 74.20%; H, 4.06%; N, 14.95%; O, 6.78%.

3-(2-(1*H*-indol-2-yl) phenyl)-2-(4-nitrophenyl)imidazo[4,5-*b*]indole (3f):

IR (KBr, cm^{-1}) *v*: 3339.16 (N-H, str.), 3037.49 (Ar C-H, str.), 1609.67 (C=N, Aromatic, str.), 1556.68 (C=C, str.), 1513.23 (N=O, str.), 1308.17 (C-N, Aromatic, str.), 853.08 (C-N (Nitro)str.); ^1H NMR (300 MHz, DMSO-d_6 in ppm): *δ* 6.419(s, 1H, Ar-H), 7.099-7.396 (m, 6H, Ar-H), 7.401-7.610 (m, 6H, Ar-H), 7.653-7.782 (dd, 2H, Ar-H), 8.259-8.267 (dd, 2H, Ar-H), 10.021 (s, 2H, N-H, D_2O exchangable); MS (ESI) m/z: 469.1 (100) $[M]^+$, Elemental analysis: Analyzed for $C_{29}H_{19}N_5O_2$: Found: C, 74.20%; H, 4.06%; N, 14.95%; O, 6.78%.

3-(2-(1*H*-indol-2-yl) phenyl)-2-(3,4,5-trimethoxyphenyl)imidazo[4,5-*b*]indole (3g):

IR (KBr, cm^{-1}) *v*: 3332.75 (N-H, str.), 3037.10 (Ar.C-H, str.), 2935.12 (C-H, Aliphatic, str.), 1611.56 (C=N, str.), 1585.98 (C=C, str.), 1322.17 (C-N, str.), 1220.73 (C-O-C, str.); ^1H NMR (300 MHz, DMSO-d_6 in ppm): *δ* 3.737(s, 9H, $(OCH_3)_3$), 6.415-6.447 (d, 3H, Ar-H), 7.010-7.320 (m, 5H, Ar-H), 7.347-7.501 (m, 4H, Ar-H), 7.522-7.682 (d, 3H, Ar-H), 9.969 (s, 2H, N-H, D_2O exchangable); MS (ESI) m/z: 514.5 (100) $[M]^+$, Elemental analysis: Analyzed for $C_{32}H_{26}N_4O_3$: Found: C, 74.68%; H, 5.10%; N, 10.93%; O, 9.30%.

3-(2-(1*H*-indol-2-yl) phenyl)-2-(4-dimethylaminophenyl)imidazo[4,5-*b*]indole (3h):

IR (KBr, cm^{-1}) *v*: 3347.38 (N-H, str.), 3015.29 (Ar.C-H, str.), 2946.44 (C-H, Aliphatic, str.), 1608.73 (C=N, str.), 1561.49 (C=C, str.), 1247.52 (C-N, str.); ^1H NMR (300 MHz, DMSO-d_6 in ppm): *δ* 2.850(s, 6H, Ar-$N(CH_3)_2$), 6.409 (s, 1H, Ar-H), 6.658-6.680 (dd, 2H, Ar-H), 7.002-7.398 (m, 8H, Ar-H), 7..412-7.441 (dd, 2H, Ar-H), 7.506-7.639 (m, 4H, Ar-H), 10.108 (s, 2H, N-H, D_2O exchangable); MS (ESI) m/z: 484 (100) $[M]^+$, Elemental analysis: Analyzed for $C_{31}H_{25}N_5$: Found: C, 79.66%; H, 5.41%; N, 14.95%.

3-(2-(1*H*-indol-2-yl) phenyl)-2-(4-methoxyphenyl)imidazo[4,5-*b*]indole (3i):

IR (KBr, cm^{-1}) *v*: 3341.48 (N-H, str.), 3019.24 (Ar.C-H, str.), 2942.70 (C-H, Aliphatic, str.), 1617.31 (C=N, str.), 1570.55 (C=C, str.), 1330.83 (C-N, str.), 1206.08 (C-O-C, str.); ^1H NMR (300 MHz, DMSO-d_6 in ppm): *δ* 3.739(s, 3H, Ar-OCH_3), 6.414 (s, 1H, Ar-H), 6.721-6.891 (dd, 2H, Ar-H), 7.002-7.397 (m, 8H, Ar-H), 7.412-7.526 (dd, 2H, Ar-H), 7.544-7.683 (m, 4H, Ar-H), 10.223 (s, 2H, N-H, D_2O exchangable); MS (ESI) m/z: 467.5 (100) $[M]^+$, Elemental analysis: Analyzed for $C_{30}H_{22}N_4O$: Found: C, 79.30%; H, 4.90%; N, 12.30%; O, 3.50%.

3-(2-(1*H*-indol-2-yl) phenyl)-2-(2-chlorophenyl)imidazo[4,5-*b*]indole (3j):

IR (KBr, cm^{-1}) *v*: 3340.67 (N-H, str.), 3071.03 (Ar C-H, str.), 1599.54 (C=N, str.), 1567.28 (C=C, str.), 1332.16 (C-N, str.), 813.33 (C-Cl, str.); ^1H NMR (300 MHz, DMSO-d_6 in ppm): δ 6.409 (s, 1H, Ar-H), 7.013-7.298 (m, 7H, Ar-H), 7.327-7.444 (m, 5H, Ar-H), 7.479-7.576 (d, 2H, Ar-H), 7.595-7.630 (d, 2H, Ar-H), 10.189 (s, 2H, N-H, D_2O exchangable); MS (ESI) m/z: 458.9 (100) $[M]^+$, Elemental analysis: Analyzed for $C_{29}H_{19}ClN_4$: Found: C, 75.86%; H, 4.14%; Cl, 7.70%; N, 12.23%.

3-(2-(1*H*-indol-2-yl) phenyl)-2-(3-chlorophenyl)imidazo[4,5-*b*]indole (3k):

IR (KBr, cm^{-1}) *v*: 3341.95 (N-H, str.), 3066.14 (Ar C-H, str.), 1596.78 (C=N, str.), 1564.47 (C=C, str.), 1330.09 (C-N, str.), 811.82 (C-Cl, str.); ^1H NMR (300 MHz, DMSO-d_6 in ppm): δ 6.423 (s, 1H, Ar-H), 7.002-7.295 (m, 7H, Ar-H), 7..306-7.522 (m, 6H, Ar-H), 7.549-7.691 (d, 3H, Ar-H), 10.218 (s, 2H, N-H, D_2O exchangable); MS (ESI) m/z: 458.9 (100) $[M]^+$, Elemental analysis: Analyzed for $C_{29}H_{19}ClN_4$: Found: C, 75.86%; H, 4.14%; Cl, 7.70%; N, 12.23%.

3-(2-(1*H*-indol-2-yl) phenyl)-2-(4-fluorophenyl)imidazo[4,5-*b*]indole (3l):

IR (KBr, cm^{-1}) *v*: .), 3051.17 (Ar C-H, str.), 1609.78 (C=N, str.), 1573.20 (C=C, str.), 1327.44 (C-N, str.), 884.82 (C-F, str.); ^1H NMR (300 MHz, DMSO-d_6 in ppm): δ 6.326 (s, 1H, Ar-H), 7.021-7.082 (dd, 2H, Ar-H), 7.123-7.304 (m, 6H, Ar-H), 7.375-7.396 (dd, 2H, Ar-H), 7.428-7.688 (m, 6H, Ar-H), 10.314 (s, 2H, N-H, D_2O exchangable); MS (ESI) m/z: 264.2 (100) $[M]^+$, Elemental analysis: Analyzed for $C_{29}H_{19}FN_4$: C, 78.70%; H, 4.26%; N, 12.65%.

Pharmacology

Antitubercular activity [9]

The method followed was Agar dilution method. The principle behind the activity is the inhibition of the number of colony formation. All the samples were tested against the standard antitubercular drug isoniazid. The percentage inhibition by the test compounds was calculated by the formula:

$$Percentage\ Inhibition\ (\%) = \left(1 - \frac{Nt}{Nc}\right) X\ 100$$

Nt = No. of colonies in sample containing plates.

Nc = No. of colonies in control plate.

Anticancer activity [10]

All the newly synthesized indole derivatives were screened for anticancer activity against MCF 7 (Human breast cancer) cell line by SRB (Sulphorodamine B) assay. The SRB assay possesses a colorimetric end point and is non destructive and indefinitely stable. These practical advances make the SRB assay an appropriate and sensitive assay to measure percent growth inhibition.

RESULTS AND DISCUSSION

All the newly synthesized indole derivatives were synthesized successfully in moderate to good yields. These compounds were identified on the basis of R_f values, solubility in various solvents, melting point range. These compounds were characterized by FTIR, ^1H NMR, Mass and elemental analysis. Compounds on ^1H NMR analysis, showed the presence of amino group and C-H pyrazole protons between δ 9.265-10.723 ppm and δ 6.701-7.279 ppm respectively, the FTIR analysis showed the presence of characteristic N-H, C=N and C-N peaks within the range 3313-3386 cm^{-1}, 1546-1610 cm^{-1} and 1317-1388 cm^{-1} respectively confirming the presence of the indole ring. The *N*-methylated compounds **3h** on ^1H NMR analysis showed the presence of protons of the methyl group within the range of δ 2.757-2.863 ppm, the FTIR analysis showed the presence of characteristic methyl C-H peak within the range 2889-2972 cm^{-1}.

Antitubercular activity

Amongst the tested compounds **(3a-3l)** the compounds **3h** (98 %, 12.5 µg/mL) and **3i** (98 %,12.5 µg/mL) exhibited good antitubercular activity compared to the standard drug isoniazid (99 %, 12.5 µg/mL).

The *o*-Nitro, *o*-Chloro, and *m*-Chloro compounds gave moderate to good activity compared to the other synthesized compounds.

Anticancer activity

Amongst the tested compounds **(3c-3g)** the compounds **3d**, **3e** and **3g** exhibited good anticancer activity compared to the standard drug Adriamycin. All the derivatives were tested on MCF7 cell line (breast cancer).

CONCLUSION

A series of substituted indole derivatives were synthesized by condensation of 2-(*o*-Aminophenyl)indole with various benzaldehydes to form *N*-(Arylidine)-2-(1*H* indol-2-yl)-benzenamines **(2a-2l)** which further react with isatin and ammonium acetate to give 3-[2-(1H-Indol-2-yl)phenyl]-2aryl-imidazo[4,5-*b*]indoles **(3a-3l)**. All of the synthesized compounds were characterized by IR, ^{1}H NMR, MASS and elemental analysis; these synthesized compounds were screened for antitubercular and anticancer activities. The antitubercular activity was performed by agar dilution method and the resulting data suggested that, amongst the tested compounds **(3a-3l),** the compounds 3h **(**98 %, 12.5 µg/mL) and 3i (98 %,12.5 µg/mL) exhibited good anti-tubercular activity compared to the standard drug isoniazid (99 %, 12.5 µg/mL). The *o*-Nitro, *o*-Chloro and *m*-Chloro compounds exhibited moderate to good activity compared to the other synthesized compounds. The anticancer activity was performed on MCF7 cell line (breast cancer) and the resulting data suggested that **3d**, **3e** and **3g** compounds exhibited good activity. From the screening data, it may be concluded that the compounds possessing substituents like *p*-Dimethylamino and *p*-Methoxy groups enhance the stability of the aryl portion exhibited moderate to good antitubercular activity compared to the standard drug isoniazid. The anticancer activity data suggested that compound **3d, 3e and 3g** exhibited promising anticancer activity with GI50 value less than 10.

ACKNOWLEDGEMENT

The authors are also thankful to the management of Rajiv Academy for Pharmacy, Mathura for providing the necessary facilities.

REFERENCES

1. *Abele E, Abele R, Dzenitis O, Lukevics E. Indole and isatin oximes: Synthesis, reaction and biological activity. Chemistry of Heterocyclic Compounds, 2003; 39: 3-29.*
2. *Billimoria AD, Cava MP. Chemistry of Indolo[1,2-c]quinazoline: An approach to the marine alkaloid Hinckdentine A. Journal of organic chemistry, 1994; 59: 6777-6782.*
3. *Biradar JS, Sasidhar BS, Parveen R. Synthesis, antioxidant and DNA cleavage activities of novel indole derivatives. European Journal of Medicinal Chemistry, 2010; 45: 4074-4078.*
4. *Carpita A, Ribecai A. Microwave-assisted synthesis of indole derivatives via cycloisomerization of 2-Alkylanilines in water without added catalysts, acids, or bases. Tetrahedron Letters, 2009; 50: 6877-6881.*
5. *Cravotto G, Demartin F, Palmisano G, Penoni A, Radice, T, Tollari S. Novel cyclometallated Pd(II) and Pt(II) complexes with indole derivatives and their useas catalysts in Heck reaction. Journal of Organometallic Chemistry, 2005; 690: 2017-2026.*
6. *Dandia A, Singh R, Singh D. Facile one-pot synthesis of new annulated hexacyclic ring system indeno-pyrano-furu-indoles and spiro indenopyran-indoles under microwave irradiation. Indian Journal of chemistry, 2009; 48B: 1001-1005.*
7. *Epifano F, Genovese S, Rosati O, Tagliapietra S, Pelucchini C. Ytterbium triflate catalyzed synthesis of β-functionalized indole derivatives. Tetrahedron Letters, 2011; 52: 568-571.*
8. *Fan X, Zhang Y. SmI2 mediated synthesis of 2,3-substituted indole derivatives. Tetrahedron, 2003; 59: 1917-1923.*
9. *Garcia LC, Martinez R. Synthesis and in vitro cytotoxic activity of pyrrolo[2,3-e]indole derivatives and a dihydro benzoindole analogue. European Journal of Medicinal Chemistry, 2002; 37: 261-266.*
10. *Gawad HA, Mohamed HA, Dawood KM, Badria FAR. Synthesis and antiviral activity of new indole-based heterocycles. Chemical and Pharmaceutical Bulletin, 2010; 58: 1529-1531.*