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**Der Pharma Chemica, 2015, 7(10):229-236**
*(http://derpharmachemica.com/archive.html)*

**ISSN 0975-413X**
**CODEN (USA): PCHHAX**

# A conventional synthesis and characterization of some novel substituted *N*-{(2-methyl/mercapto--1*H*-benzo[*d*]imidazol-1-yl)methyl}substituted benzenamine, *N*-{(2-methyl/mercapto--1*H*-benzo[*d*]imidazol-1-yl)(substituted phenyl)methyl}substituted benzenamine as potential cytotoxic agents

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**Abstract**

*Benzimidazoles are an important group of heterocyclic compounds which are biologically active. The main objective to synthesized a new series of N-{(2-Methyl/Mercapto-1H-benzo[d]imidazol-1-yl)methyl}substituted benzenamine* ***(1a-1c) & (3a-3c),*** *N-{(2-Methyl/Mercapto-1H-benzo[d]imidazol-1-yl)(substituted phenyl)methyl} substituted benzenamine* ***(2a-2d) & (4a-4d)*** *were synthesized. All the newly synthesized compounds were characterized by FTIR,* $^{1}$*H NMR, mass spectral analysis and elemental analysis. All the compounds were screened for anticancer activity against A549 cell line by SRB assay of which compounds* ***1a, 1b, 2a, 2b, 2c, 2d, 3a, 3b, 4a, 4b, 4c*** *and* ***4d*** *showed promising cytotoxic potential.*

**Keywords:** Cytotoxicity, benzimidazole, SRB, anticancer, A549.

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## INTRODUCTION

The Benzimidazole ring system is a useful structural moiety found in the numerous biologically active compounds. The development and design of new synthetic approach is a challenge for the organic chemist. Therefore to meet the facile results of these tough challenges, benzimidazole nucleus was being considered. Benzimidazole is a very important pharmacophore in drug discovery, and its derivatives are used as an important class of bioactive molecules in the field of new drug development [1]. Benzimidazole nucleus has capability to inhibit the growth of various bacteria, yeast, fungi, protozoa and helminthes [2]. Benzimidazole are the versatile pharmacophore havig various biological activities like antibacterial [3], antifungal [4], Anthelmintic [5], antiprotozoal [6], anticoagulant [7], analgesic, anti-inflammatory [8], anticancer [9], anti-HIV [10], antiulcer [11], antiviral [12], antihistaminic [13], antioxidant [14], anticonvulsant [15], hypolipidemic activities [16], etc. activities and have wide applications as pharmaceutical and agrochemical agents. There are some synthetic compounds with benzimidazole nucleus used for anticancer activities. SAR studies revealed that the novel substituted *N*-1 as well as 2-substituted benzimidazoles played an integral role for the increase in cytotoxic potential. In view of these observations and in continuation of our research to develop better and potent anticancer agents, it was contemplated to synthesize a series of some novel compounds possessing benzimidazole moiety.

**Table 1: Results for cytotoxicity by SRB assay in A549 cell line**

| S. No. | Compound | $CTC_{50}$ (µg/ml) |
|---|---|---|
| 1 | **1a** | 144 |
| 2 | **1b** | >200 |
| 3 | **1c** | 110 |
| 4 | **2a** | 123 |
| 5 | **2b** | 172 |
| 6 | **2c** | 125 |
| 7 | **2d** | 138 |
| 8 | **3a** | 105 |
| 9 | **3b** | >200 |
| 10 | **3c** | 109 |
| 11 | **4a** | 136 |
| 12 | **4b** | 117 |
| 13 | **4c** | 119 |
| 14 | **4d** | 113 |

## MATERIALS AND METHODS

### Experimental

Melting points were taken in open capillary tube and were uncorrected. The purity of all the newly synthesized compounds was checked by TLC on silica gel G plates. The solvent system was chloroform: methanol: 1: 1. The UV spectra were recorded on a SHIMADZU spec-1700, IR spectra on a SHIMADZU 8400S spectrophotometer, $^1$H NMR spectra on a Brucker DRX 300 in DMSO using TMS (Tetramethyl silane) as an internal standard and Mass spectrum on an MS-ESI (SHIMADZU-2010 AT, software class VP). Elemental analysis was carried out on elemental vario EL III Carlo Erba 1108.

### Procedure for synthesis of 2-Methyl-1*H*-benzo[*d*]imidazole

*o*-Phenylenediamine dihydrochloride (10 mmol), water 5 ml and acetic acid (30 mmol) were added to the flask and the reaction mixture was refluxed for 1 hr. The flask was then removed, cooled at room temperature and conc. ammonia solution was added slowly with constant stirring until the reaction mixture become alkaline. The product was precipitated out, washed with ice cold water, filtered, dried and recrystallized from aqueous ethanol.

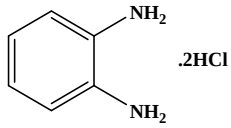
### Procedure for Synthesis of *N*-{(2-Methyl/Mercapto-1*H*-benzo[*d*]imidazol-1-yl)methyl}substituted benzenamine (1a-1c) & (3a-3c), *N*-{(2-Methyl/Mercapto-1*H*-benzo[*d*]imidazol-1-yl)(substituted phenyl) methyl}substituted benzenamine (2a-2d) & (4a-4d)

Eqimolar quantities of compound 2-Methyl/Mercapto benzimidazole (10 mmol), Substituted aryl amine (10 mmol) and formaldehyde/Substituted benzaldehyde (10 mmol) were taken in 15 ml. of ethanol and refluxed for 10-24 hrs. On cooling, the product formed was filtered, dried and purified by recrystallization with 40% aq. Ethanol.

**Scheme of work**

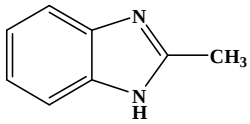
**Scheme-1:**

**Step-1:**



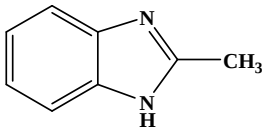

***o*-Phenylenediamine dihydrochloride**

**Reflux for 1 hr.** **Glacial acetic acid Water**



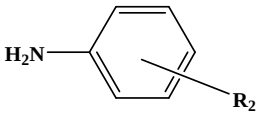

**2-Methyl-1*H*-benzo[*d*]imidazole**

**Step-2:**




**2-Methyl-1*H*-benzo[*d*]imidazole**

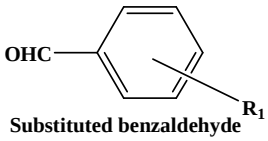
**HCHO Formaldehyde**



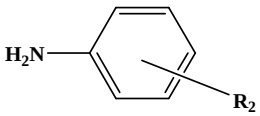

**Substituted aryl amine**

**Reflux for 10-24 hr.**

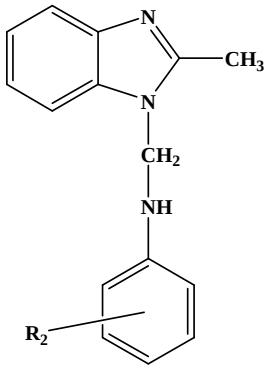
**Ethanol**




**Substituted benzaldehyde**

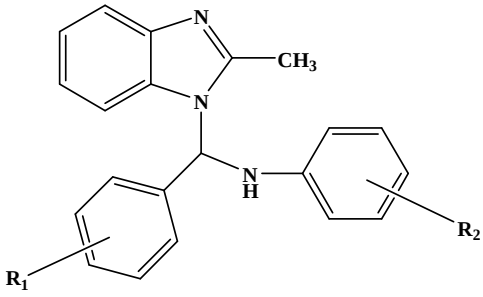



**Substituted aryl amine**




***N*-{(2-Methyl-1*H*-benzo[*d*]imidazol-1-yl) methyl}substituted benzenamine**

**(1a)-(1c)**




***N*-{(2-Methyl-1*H*-benzo[*d*]imidazol-1-yl) (substituted phenyl)methyl}substituted benzenamine**

**(2a)-(2d)**

**Scheme-2:**

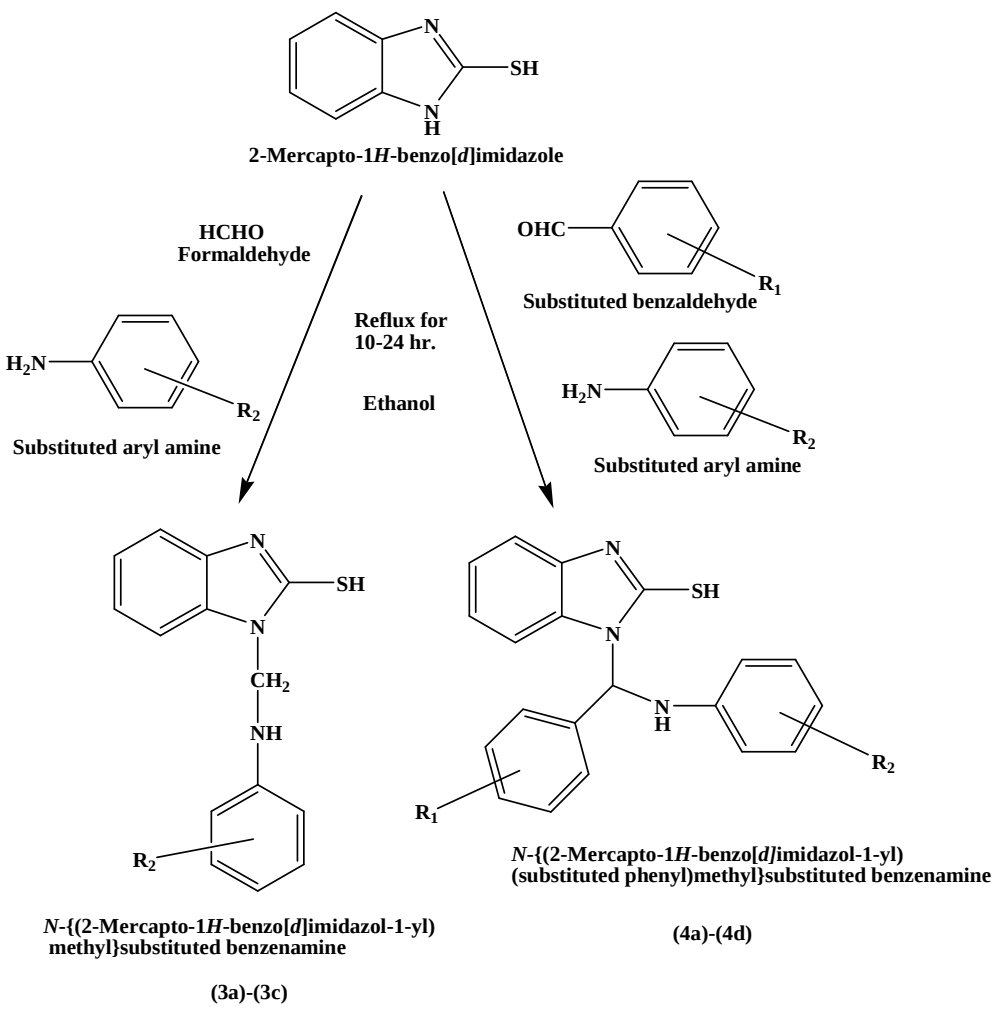



**Table 2: List of substituent**

| Compound No. | $R_1$ | $R_2$ |
|---|---|---|
| **1a** | - | *p*-$NO_2$ |
| **1b** | - | *m*-$OCH_3$ |
| **1c** | - | *p*-Cl |
| **2a** | *p*-OH | *p*-$NO_2$ |
| **2b** | *p*-F | *p*-$NO_2$ |
| **2c** | *m*-$OCH_3$ | *p*-$NO_2$ |
| **2d** | *m*-$NO_2$ | *p*-Cl |
| **3a** | - | *p*-$NO_2$ |
| **3b** | - | *m*-$OCH_3$ |
| **3c** | - | *p*-Cl |
| **4a** | *p*-OH | *p*-$NO_2$ |
| **4b** | *p*-F | *p*-$NO_2$ |
| **4c** | *m*-$OCH_3$ | *p*-$NO_2$ |
| **4d** | *m*-$NO_2$ | *p*-Cl |

**Compound detail**

***N*-{(2'-Methyl-1*H*-benzo[*d*]imidazol-1-yl)methyl}-4-nitrobenzenamine (1a):**

Yield: 81.41 %, m.p. 144-146°C; Elemental analysis Calcd for $C_{15}H_{14}N_4O_2$: C, 63.82; H, 5.00; N, 19.85. Found: C, 63.80; H, 5.01; N, 19.81 %; FTIR (KBr, $\nu_{max}$, $cm^{-1}$): 3365 (N-H str. (2°amine)), 3051 (Aromatic C-H str.), 2935 (Aliphatic C-H str.), 1677 (C=N str.), 1630 (Aromatic C-----C str.), 1585 (Aromatic C-C str.), 1419 (N-O str.), 1315

(Aromatic C-N str.), 1174 (Aliphatic C-N str.), 813 (C-H *p*-disubstituted benzene (def.)); $^1$H NMR **(**DMSO-$d_6$**)** δ(ppm)**:** 2.70 (s, 3H, $CH_3$), 4.10 (s, 1H, N-H, $D_2O$ exchangeable), 4.32 (s, 2H, $CH_2$), 7.203-7.249 (t, 2H, Ar–H), 7.410-7.425 (d, 2H, Ar–H), 7.501-7.527 (d, 2H, Ar–H), 7.854-7.895 (d, 2H, Ar–H); MS (ESI) m/z [% rel. abundance]: 282 (100) $[M]^+$, 283 (16) $[M+1]^+$.

**3-Methoxy-*N*-{(2'-methyl-1*H*-benzo[*d*]imidazol-1-yl)methyl}benzenamine (1b):**
Yield: 75.7 %, m.p. 164-166°C; Elemental analysis Calcd for $C_{16}H_{17}N_3O$: C, 71.89; H, 6.41; N, 15.72. Found: C, 71.87; H, 6.40; N, 15.69 %; FTIR (KBr, $\nu_{max,}$ $cm^{-1}$): 3350 (N-H str. (2°amine)), 3056 (Aromatic C-H str.), 2921 (Aliphatic C-H str.), 1687 (C=N Str.), 1620 (Aromatic C⋯C str.), 1271 (Aromatic C-N str.), 1209 (Aliphatic C-N str.), 1084 (C-O-C str.), 693 (C-H *m*-disubstituted benzene (def.)); $^1$H NMR **(**DMSO-$d_6$**)** δ(ppm)**:** 2.320 (s, 3H, $CH_3$), 3.734 (s, 3H, $OCH_3$), 4.122 (s, 1H, N-H, $D_2O$ exchangeable), 4.601 (s, 2H, $CH_2$), 6.080-6.093 (d, 1H, Ar–H), 6.408 (s, 1H, Ar–H), 6.700-6.777 (t, 1H, Ar-H), 6.940-6.957 (d, 1H, Ar-H), 7.260-7.283 (t, 2H, Ar–H), 7.509-7.525 (d, 2H, Ar–H); MS (ESI) m/z [% rel. abundance]: 267 (100) $[M]^+$, 268 (18) $[M+1]^+$.

**4-Chloro-*N*-{(2'-methyl-1*H*-benzo[*d*]imidazol-1-yl) methyl} benzenamine (1c):**
Yield: 63.71 %, m.p. 180-182°C; Elemental analysis Calcd for $C_{15}H_{14}N_3Cl$: C, 66.30; H, 5.19; N, 15.12; Cl, 13.05. Found: C, 66.27; H, 5.18; N, 15.09; Cl, 13.01 %; FTIR (KBr, $\nu_{max,}$ $cm^{-1}$): 3356 (N-H str. (2°amine)), 3037 (Aromatic C–H str.), 2935 (Aliphatic C-H str.), 1664 (C=N str.), 1623 (C=C ring str.), 1282 (Aromatic C-N str.), 1164 (Aliphatic C-N str.), 1092 $cm^{-1}$ (Aromatic C-Cl str.), 829 (C-H *p*-disubstituted benzene (def.)); $^1$H NMR **(**DMSO-$d_6$**)** δ(ppm)**:** 2.102 (s, 3H, $CH_3$), 4.00 (s, 1H, N-H, $D_2O$ exchangeable), 4.410 (s, 2H, $CH_2$), 7.053-7.109 (t, 2H, Ar–H), 7.210-7.235 (d, 2H, Ar–H), 7.421-7.439 (d, 2H, Ar–H), 7.654-7.690 (d, 2H, Ar–H); MS (ESI) m/z [% rel. abundance]: 271 (100) $[M]^+$, 272 (16) $[M+1]^+$, 273 (27) $[M+2]^+$.

**4-{(4'-Nitrophenylamino)(2''-methyl-1*H*-benzo[*d*]imidazol-1-yl)methyl}phenol (2a):**
Yield: 78.2 %, m.p. 178-180°C; Elemental analysis Calcd for $C_{21}H_{18}N_4O_3$: C, 67.37; H, 4.85; N, 14.96. Found: C, 67.35; H, 4.84; N, 14.93 %; FTIR (KBr, $\nu_{max,}$ $cm^{-1}$): 3640 (O-H str.), 3361 (N-H str. (2°amine)), 3072 (Aromatic C-H str.), 2896 (Aliphatic C-H str.), 1675 (C=N Str.), 1625 (Aromatic C⋯C str.), 1483 (N-O Str.), 1313 (Aromatic C-N str.), 1186 (Aliphatic C-N str.), 840 (C-H *p*-disubstituted benzene (def.)); $^1$H NMR **(**DMSO-$d_6$**)** δ(ppm)**:** 2.803 (s, 3H, $CH_3$), 4.502 (s, 1H, N-H, $D_2O$ exchangeable), 5.20 (s, 1H, OH, $D_2O$ exchangeable), 6.106 (s, 1H, CH), 6.603-6.625 (d, 2H, Ar–H), 6.804-6.829 (d, 2H, Ar–H), 6.904-6.983 (t, 2H, Ar–H), 6.259-6.285 (d, 2H, Ar-H), 7.310-7.329 (d, 2H, Ar–H), 7.901-7.925 (d, 2H, Ar–H); MS (ESI) m/z [% rel. abundance]: 374 (100) $[M]^+$, 375 (16) $[M+1]^+$.

***N*-{(4'-Fluorophenyl)(2''-methyl-1*H*-benzo[*d*]imidazol-1-yl)methyl}-4-nitrobenzenamine (2b):**
Yield: 85.3 %, m.p. 134-136°C; Elemental analysis Calcd for $C_{21}H_{17}FN_4O_2$: C, 67.01; H, 4.55; F, 5.05; N, 14.89. Found: C, 67.02; H, 4.52; F, 5.02; N, 14.86 %; FTIR (KBr, $\nu_{max,}$ $cm^{-1}$): 3357 (N-H str. (2°amine)), 3055 (Aromatic C-H str.), 2889 (Aliphatic C-H str.), 1680 (C=N str.), 1616 (Aromatic C⋯C str.), 1431 (N-O Str.), 1313 (Aromatic C-N str.), 1205 (C-F Str.), 1103 (Aliphatic C-N str.), 820 (C-H *p*-disubstituted benzene (def.)); $^1$H NMR **(**DMSO-$d_6$**)** δ(ppm)**:** 2.401 (s, 3H, $CH_3$), 4.00 (s, 1H, N-H, $D_2O$ exchangeable), 6.10 (s, 1H, CH), 6.920-6.957 (d, 2H, Ar–H), 7.240-7.248 (d, 2H, Ar–H), 7.303-7.399 (t, 2H, Ar–H), 7.512-7.528 (d, 2H, Ar-H), 7.802-7.815 (d, 2H, Ar–H), 7.902-7.957 (d, 2H, Ar–H); MS (ESI) m/z [% rel. abundance]: 376 (100) $[M]^+$, 377 (12) $[M+1]^+$, 378 (24) $[M+2]^+$.

***N*-{(3-Methoxyphenyl)(2''-methyl-1*H*-benzo[*d*]imidazol-1-yl)methyl}-4-nitrobenzenamine (2c):**
Yield: 83.5 %, m.p. 152-154°C; Elemental analysis Calcd for $C_{22}H_{20}N_4O_3$ : C, 68.03; H, 5.19; N, 14.42. Found: C, 68.01; H, 5.16; N, 14.41 %; FTIR (KBr, $\nu_{max,}$ $cm^{-1}$): 3361 (N-H str. (2°amine)), 3062 (Aromatic C-H str.), 2898 (Aliphatic C-H str.), 1687 (C=N str.), 1610 (Aromatic C⋯C str.), 1476 (N-O str.), 1313 (Aromatic C-N str.), 1190 (C-O-C str.), 1125 (Aliphatic C-N str.), 812 (C-H *p*-disubstituted benzene (def.)), 705 (C-H *m*-disubstituted benzene (def.)); $^1$H NMR **(**DMSO-$d_6$**)** δ(ppm)**:** 2.203 (s, 3H, $CH_3$), 3.734 (s, 3H, $OCH_3$), 4.312 (s, 1H, N-H, $D_2O$ exchangeable), 6.280 (s, 1H, CH), 6.093-6.548 (d, 1H, Ar–H), 6.600 (s, 1H, Ar–H), 6.738-6.840 (t, 1H, Ar–H), 6.957-7.060(d, 1H, Ar-H), 7.269-7.299 (t, 2H, Ar–H), 7.305-7.359 (d, 2H, Ar–H), 7.505-7.529 (d, 2H, Ar–H), 7.925-7.945 (d, 2H, Ar–H); MS (ESI) m/z [% rel. abundance]: 388 (100) $[M]^+$, 389 (16) $[M+1]^+$.

***N*-{(3'-Nitrophenyl)(2''-methyl-1*H*-benzo[*d*]imidazol-1-yl)methyl}-4-chlorobenzenamine (2d):**
Yield: 68.97 %, m.p. 218-220°C; Elemental analysis Calcd for $C_{21}H_{17}N_4O_2Cl$: C, 64.21; H, 4.36; N, 14.26; Cl, 9.02. Found: C, 64.19; H, 4.35; N, 14.23; Cl, 9.01%; FTIR (KBr, $\nu_{max,}$ $cm^{-1}$): 3380 (N-H str. (2°amine)), 3049 (Aromatic

C-H str.), 2910 (Aliphatic C-H str.), 1664 (C=N str.), 1610 (Aromatic C⋯C str.), 1446 (N-O str.), 1332 (Aromatic C-N str.), 1226 (Aliphatic C-N str.), 1092 (C-Cl str.), 834 (C-H *p*-Disubstituted benzene (def.)), 691 (C-H *m*-disubstituted benzene (def.)); $^1$H NMR **(**DMSO-$d_6$**)** δ(ppm)**:** 2.300 (s, 3H, $CH_3$), 4.207 (s, 1H, N-H, $D_2O$ exchangeable), 6.103 (s, 1H, CH), 7.103-7.179 (t, 2H, Ar–H), 7.210-7.243 (d, 2H, Ar–H), 7.302-7.349 (d, 2H, Ar–H), 7.410-7.435 (d, 1H, Ar-H), 7.501-7.584 (t, 1H, Ar–H), 7.605-7.641 (d, 2H, Ar–H), 7.907 (s, 1H, Ar–H), 7.914-8.025 (d, 1H, Ar–H); MS (ESI) m/z [% rel. abundance]: 392 (100) $[M]^+$, 393 (12) $[M+1]^+$, 394 (23) $[M+2]^+$.

**1-{(4'-Nitrophenylamino) methyl}-1*H*-benzo[*d*]imidazole-2-thiol (3a):**
Yield: 87.7 %, m.p. 236-238°C; Elemental analysis Calcd for $C_{14}H_{12}N_4O_2S$ : C, 55.99; H, 4.03; N, 18.65; S, 10.68. Found: C, 55.97; H, 4.01; N, 18.63; S, 10.62 %; FTIR (KBr, $\nu_{max,}$ $cm^{-1}$): 3370 (N-H str. (2°amine)), 3075 (Aromatic C-H str.), 2925 (Aliphatic C-H str.), 2559 (S-H Str.), 1635 (Aromatic C⋯C Str.), 1623 (C=N Str.), 1593 (Aromatic C-C str.), 1454 (N-O Str.), 1325 (Aromatic C-N str.), 1180 (Aliphatic C-N str.), 806 (C-H *p*-disubstituted benzene (def.)); $^1$H NMR **(**DMSO-$d_6$**)** δ(ppm)**:** 3.007 (s, 1H, SH, $D_2O$ exchangeable ), 4.000 (s, 1H, N-H, $D_2O$ exchangeable), 4.502 (s, 2H, $CH_2$), 7.215-7.269 (t, 2H, Ar–H), 7.304-7.325 (d, 2H, Ar–H), 7.610-7.630 (d, 2H, Ar–H), 7.904-7.923 (d, 2H, Ar–H); MS (ESI) m/z [% rel. abundance]: 300 (100) $[M]^+$, 301 (15) $[M+1]^+$.

**1-{(3'-Methoxyphenylamino) methyl}-1*H*-benzo[*d*]imidazole-2-thiol (3b):**
Yield: 83.8 %, m.p. 220-222°C; Elemental analysis Calcd for $C_{15}H_{15}N_3OS$: C, 63.13; H, 5.30; N, 14.73; S, 11.24. Found: C, 63.11; H, 5.28; N, 14.71; S, 11.22 %; FTIR (KBr, $\nu_{max,}$ $cm^{-1}$): 3364 (N-H str. (2°amine)), 3072 (Aromatic C-H str.), 2879 (Aliphatic C-H str.), 2572 (S-H Str.), 1682 (C=N str.), 1614 (Aromatic C⋯C str.), 1274 (Aromatic C-N str.), 1190 (Aliphatic C-N Str.), 1107 (C-O-C Str.), 710 (C-H *m*-Disubstituted benzene (def.)); $^1$H NMR **(**DMSO-$d_6$**)** δ(ppm)**:** 3.220 (s, 1H, SH, $D_2O$ exchangeable), 3.730 (s, 3H, $OCH_3$), 4.000 (s, 1H, N-H, $D_2O$ exchangeable), 4.302 (s, 2H, $CH_2$), 6.110-6.133 (d, 1H, Ar–H), 6.408 (s, 1H, Ar–H), 6.701-6.767 (t, 1H, Ar-H), 6.940-6.953 (d, 1H, Ar-H),7.310-7.364 (t, 2H, Ar–H), 7.733-7.765 (d, 2H, Ar–H); MS (ESI) m/z [% rel. abundance]: 285 (100) $[M]^+$, 286 (15) $[M+1]^+$.

**1-{(4'-Chlorophenylamino) methyl}-1*H*-benzo[*d*]imidazole-2-thiol (3c):**
Yield: 83.4 %, m.p. 252-254°C; Elemental analysis Calcd for $C_{14}H_{12}N_3SCl$: C, 58.03; H, 4.17; N, 14.50; S, 11.07; Cl, 12.23. Found: C, 58.01; H, 4.15; N, 14.47; S, 11.02; Cl, 12.21 %; FTIR (KBr, $\nu_{max,}$ $cm^{-1}$): 3362 (N-H str. (2°amine)), 3071 (Aromatic C–H str.), 2952 (Aliphatic C-H str.), 2571 (S-H str.), 1683 (C=N str.), 1602 (C=C ring str.), 1317 (Aromatic C-N str.), 1182 (Aliphatic C-N str.), 1095 $cm^{-1}$ (Aromatic -Cl str.), 835 (C-H *p*-disubstituted (def.)); $^1$H NMR **(**DMSO-$d_6$**)** δ(ppm)**:** 3.102 (s, 1H, SH, $D_2O$ exchangeable), 4.010 (s, 1H, N-H, $D_2O$ exchangeable), 4.402 (s, 2H, $CH_2$), 7.043-7.103 (t, 2H, Ar–H), 7.216-7.243 (d, 2H, Ar–H), 7.401-7.435 (d, 2H, Ar–H), 7.509-7.524 (d, 2H, Ar–H); MS (ESI) m/z [% rel. abundance]: 289 (100) $[M]^+$, 290 (15) $[M+1]^+$, 291 (26) $[M+2]^+$.

**4-{(4'-Nitrophenylamino)(2''-mercapto-1*H*-benzo[*d*]imidazol-1-yl)methyl}phenol (4a):**
Yield: 69.6 %, m.p. 210-212°C; Elemental analysis Calcd for $C_{20}H_{16}N_4O_3S$ : C, 61.21; H, 4.11; N, 14.28; S, 8.17. Found: C, 61.18; H, 4.09; N, 14.25; S, 8.14 %; FTIR (KBr, $\nu_{max,}$ $cm^{-1}$): 3620 (O-H str.), 3359 (N-H str. (2°amine)), 3063 (Aromatic C-H str.), 2893 (Aliphatic C-H str.), 2570 (S-H Str.), 1661 (C=N str.), 1631 (Aromatic C⋯C str.), 1469 (N-O Str.), 1303 (Aromatic C-N str.), 1176 (Aliphatic C-N str.), 823 (C-H *p*-disubstituted benzene (def.)); $^1$H NMR **(**DMSO-$d_6$**)** δ(ppm)**:** 3.401 (s, 1H, SH, $D_2O$ exchangeable), 4.300 (s, 1H, N-H, $D_2O$ exchangeable), 5.000 (s, 1H, OH, $D_2O$ exchangeable), 6.206 (s, 1H, CH), 6.603-6.625 (d, 2H, Ar–H), 6.807-6.823 (d, 2H, Ar–H), 7.156-7.181 (d, 2H, Ar–H), 7.309-7.385 (t, 2H, Ar-H), 7.479-7.505 (d, 2H, Ar–H), 7.810-7.844 (d, 2H, Ar–H); MS (ESI) m/z [% rel. abundance]: 392 (100) $[M]^+$, 393 (12) $[M+1]^+$.

**1-{(4'-Nitrophenylamino)(4''-fluorophenyl)methyl}-1*H*-benzo[*d*]imidazole-2-thiol (4b):**
Yield: 84.76 %, m.p. 240-242°C; Elemental analysis Calcd for $C_{20}H_{14}FN_3O_2S$ : C, 63.31; H, 3.72; F, 5.03; N, 11.08; S, 8.45. Found: C, 63.16; H, 3.53; F, 5.01; N, 11.02; S, 8.20; %; FTIR (KBr, $\nu_{max,}$ $cm^{-1}$): 3386 (N-H str. (2°amine)), 3089 (Aromatic C-H str.), 2887 (Aliphatic C-H str.), 2557 (S-H Str.), 1685 (C=N str.), 1620 (Aromatic C⋯C str.), 1413 (N-O str.), 1299 (Aromatic C-N str.), 1208 (C-F str.), 1145 (Aliphatic C-N str.), 820 (C-H *p*-disubstituted benzene (def.)); $^1$H NMR **(**DMSO-$d_6$**)** δ(ppm)**:** 3.100 (s, 1H, SH, $D_2O$ exchangeable), 4.010 (s, 1H, N-H, $D_2O$ exchangeable), 6.109 (s, 1H, CH), 6.932-6.951 (d, 2H, Ar–H), 7.210-7.283 (t, 2H, Ar–H), 7.311-7.334 (d, 2H, Ar–H), 7.602-7.629 (d, 2H, Ar-H), 7.832-7.853 (d, 2H, Ar–H), 7.941-7.959 (d, 2H, Ar–H); MS (ESI) m/z [% rel. abundance]: 394 (100) $[M]^+$, 395 (12) $[M+1]^+$ , 396 (25) $[M+2]^+$.

**1-{(4'-Nitrophenylamino)(3''-methoxyphenyl)methyl}-1*H*-benzo[*d*]imidazole-2-thiol (4c):**
Yield: 80.7 %, m.p. 226-228°C; Elemental analysis Calcd for $C_{21}H_{17}N_3O_3S$ : C, 64.43; H, 4.38; N, 10.73; S, 8.19. Found: C, 64.41; H, 4.36; N, 10.71; S, 8.16 %; FTIR (KBr, $\nu_{max,}$ $cm^{-1}$): 3361 (N-H str. (2°amine)), 3040 (Aromatic C-H str.), 2881 (Aliphatic C-H str.), 2578 (S-H str.), 1675 (C=N str.), 1618 (Aromatic C⋯C str.), 1467 (N-O str.), 1313 (Aromatic C-N str.), 1186 (C-O-C str.), 1110 (Aliphatic C-N str.), 820 (C-H *p*-disubstituted benzene (def.)), 708 (C-H *m*-disubstituted benzene (def.)); $^1$H NMR **(**DMSO-$d_6$**)** δ(ppm)**:** 3.200 (s, 1H, SH, $D_2O$ exchangeable ), 3.704 (s, 3H, $OCH_3$), 4.212 (s, 1H, N-H, $D_2O$ exchangeable), 6.120 (s, 1H, CH), 6.193-6.528 (d, 1H, Ar–H), 6.600 (s, 1H, Ar–H), 6.728-6.900 (t, 1H, Ar–H), 6.927-7.030 (d, 1H, Ar-H), 7.209-7.289 (t, 2H, Ar–H), 7.305-7.399 (d, 2H, Ar–H), 7.605-7.629 (d, 2H, Ar–H), 7.813-7.845 (d, 2H, Ar–H); MS (ESI) m/z [% rel. abundance]: 406 (100) $[M]^+$, 407 (12) $[M+1]^+$.

**1-{(4'-Chlorophenylamino)(3''-nitrophenyl)methyl}-1*H*-benzo[*d*]imidazole-2-thiol (4d):**
Yield: 73.05 %, m.p. 190-192°C; Elemental analysis Calcd for $C_{20}H_{15}N_4O_2SCl$ : C, 58.46; H, 3.68; N, 13.64; S, 7.80; Cl, 8.63. Found: C, 58.45; H, 3.63; N, 13.62; S, 7.76; Cl, 8.61 %; FTIR (KBr, $\nu_{max,}$ $cm^{-1}$): 3380 (N-H str. (2°amine)), 3080 (Aromatic C-H str.), 2958 (Aliphatic C-H str.), 2559 (S-H Str.), 1685 (C=N str.), 1602 (Aromatic C⋯C str.), 1421 (N-O str.), 1326 (Aromatic C-N str.), 1180 (Aliphatic C-N str.), 1090 (C-Cl str.), 812 (C-H *p*-disubstituted benzene (def.)), 703 (C-H *m*-disubstituted benzene (def.)); $^1$H NMR **(**DMSO-$d_6$**)** δ(ppm)**:** 3.102 (s, 1H, SH, $D_2O$ exchangeable), 4.102 (s, 1H, N-H, $D_2O$ exchangeable), 6.107 (s, 1H, CH), 7.103-7.189 (t, 2H, Ar–H), 7.212-7.240 (d, 2H, Ar–H), 7.303-7.342 (d, 1H, Ar–H), 7.401-7.437 (d, 2H, Ar-H), 7.510-7.574 (t, 1H, Ar–H), 7.705-7.731 (d, 2H, Ar–H), 7.907 (s, 1H, Ar–H), 7.915-8.035 (d, 1H, Ar–H); MS (ESI) m/z [% rel. abundance]: 410 (100) $[M]^+$, 411 (12) $[M+1]^+$ , 412 (25) $[M+2]^+$.

## RESULTS AND DISCUSSION

The novel benzimidazole derivatives were synthesized successfully in moderate to good yields. The newly synthesized compounds were identified on the basis of $R_f$ values; melting point range; solubility in different solvents; FTIR, $^1$H NMR, mass spectral analysis and elemental analysis. All the newly synthesized benzimidazole derivatives were screened for anticancer activity against A549 (Human lung adenocarcinoma epithelial) cell line by SRB (Sulforhodamine B) assay.

**Anticancer screening**:
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 drug-induced cytotoxicity.

**Principle:** SRB (Kiton red) is a fluorescent dye. Under mild acidic conditions, SRB binds to protein basic amino acid residues in Trichloro acetic acid (TCA) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of at least two orders of magnitude. Colour development in SRB assay is rapid, stable and visible. The developed colour can be measured over a broad range of visible wavelength in either a spectrophotometer or a 96 well plate reader [17].

## CONCLUSION

From the cytotoxicity data, it was concluded that the compounds possessing nitro and methoxy substitution *viz*. **1a, 1b, 2a, 2b, 2c, 2d, 3a, 3b, 4a, 4b, 4c** and **4d** exhibited highest degree of inhibition against A549 cell line and this fact warrants further investigation of these compounds as promising anticancer agents.

**Acknowledgement**
The authors are grateful thankful to the Department of Pharmaceutical Chemistry of Rajiv Academy for Pharmacy, Mathura (U.P.) for providing facilities for this research work. The authors are also thankful to Central Drug Research Institute (CDRI), Lucknow and IIT, New Delhi (India) for providing spectral data and elemental analysis of the synthesized compounds.

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