



# Metronidazole aryloxy, carboxy andazole derivatives: Synthesis, anti-tumor activity, QSAR, molecular docking and dynamics studies

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## ABSTRACT

A series of novel metronidazole aryloxy, carboxy andazole derivatives has been synthesized and their cytotoxic activities on three cancer cell lines were evaluated by MTT assay. Compounds **4m**, **4l** and **4d** showed the most potent cytotoxic activity (IC<sub>50</sub>s less than 100 µg/mL). Apoptosis was also detected for these compounds by flow cytometry. Docking studies were performed in order to propose the probable target protein. In the next step, molecular dynamics simulation was carried out on the proposed target protein, focal adhesion kinase (FAK, PDB code: 2ETM), bound to compound **4m**. As, **4m** showed a potent cytotoxic activity and an acceptable apoptotic effect, it can be a potential anticancer candidate that may work through inhibition of FAK.

## 1. Introduction

The Cancer is a major health problem worldwide. In spite of being the choice method of treatment, chemotherapy is still highly inadequate and there is a massive demand for discovering new compounds with more potent anticancer activity with fewer side effects<sup>1,2</sup>. To reach this aim, many researchers are attempting to synthesis new compounds which specifically affect molecular pathways in tumor cells. Metronidazole derivatives are a class of nitroimidazole compounds with notable biological activities such as antiprotozoal,<sup>3</sup> antibacterial,<sup>4</sup> antifungal,<sup>5</sup> anti-HIV,<sup>6</sup> antimycobacterial,<sup>7</sup> and anti-tumor activity<sup>8–12</sup>. The anti-tumor activity, in particular, has attracted much attention due to their affinity for penetration and accumulation in hypoxic tumors<sup>13</sup>. Nitroimidazoles can act as oxygen mimics especially in hypoxic cells, so they can be used during irradiation to fix the radiation-induced damage in DNA or other macromolecules<sup>14</sup>. Angiogenesis plays a very crucial role in tumor growth and metastasis. Therefore, reducing the levels of angiogenic factors can be considered as a potential therapeutic method for inhibition of tumor- specific angiogenesis. It is reported that some

derivatives of 2-Nitroimidazole inhibited tumor- specific angiogenesis by blocking the production of angiogenic factors<sup>15</sup>.

Additionally, there are other molecules proposed as targets of metronidazole derivatives. In 2010, Qian et al.<sup>8</sup> synthesized a series of cinnamic acid metronidazole ester derivatives. The biological activity evaluation of the compounds indicated that some were potent inhibitors of EGFR and HER-2. Also, in 2011, Luo et al.<sup>10</sup> synthesized a series of metronidazole acid acyl sulfonamide. After biological activity assessment, it was revealed that these compounds were potent anticancer agents and potential EGFR tyrosine kinase as well as HER-2 inhibitors. Later, a series of 2-styryl-5-nitroimidazole derivatives were introduced by Duan et al. as focal adhesion kinase (FAK) inhibitors with anticancer activity<sup>11</sup>. Another investigation by this research group showed that 1-indolyl acetates 5-nitroimidazole targeted tubulin polymerization and displayed a strong antiproliferative activity<sup>12</sup>. Some of the pyridine ring containing metronidazole derivatives synthesized by Yong et al. also showed quite good anticancer activities<sup>16</sup>.

In this study, we describe the synthesis and quantitative structure activity relationship of a series of 26 novel aryloxy, carboxy, andazole

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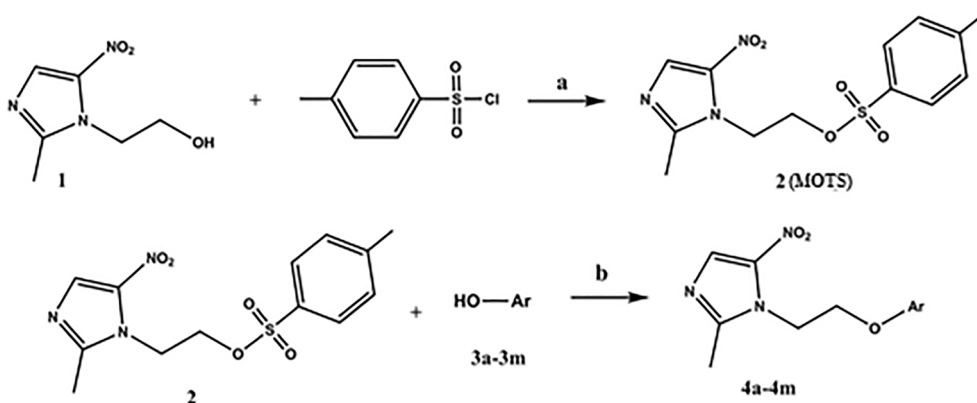
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**Scheme 1.** Reagents and conditions: (a) CHCl<sub>3</sub>, Pyridine, 0 °C, 24 h; (b) K<sub>2</sub>CO<sub>3</sub>, DMF, 80 °C, 24 h.

derivatives of metronidazole. Biological evaluation was also carried out for screening the potential cytotoxic activity by MTT assay. The apoptosis study of three more potent of these compounds was performed by flow cytometry to understand their groundwork mechanism of action. Molecular docking and dynamics studies were also done based on the structure similarity of these compounds to previously reported compounds that were potent EGFR tyrosine kinase inhibitors<sup>10</sup>.

## 2. Results and discussion

### 2.1. Chemistry

A series of novel metronidazole aryloxy and carboxy derivatives (4a-4m, 4n-4u) were synthesized according to the scheme 1 and the route outline in scheme 2.

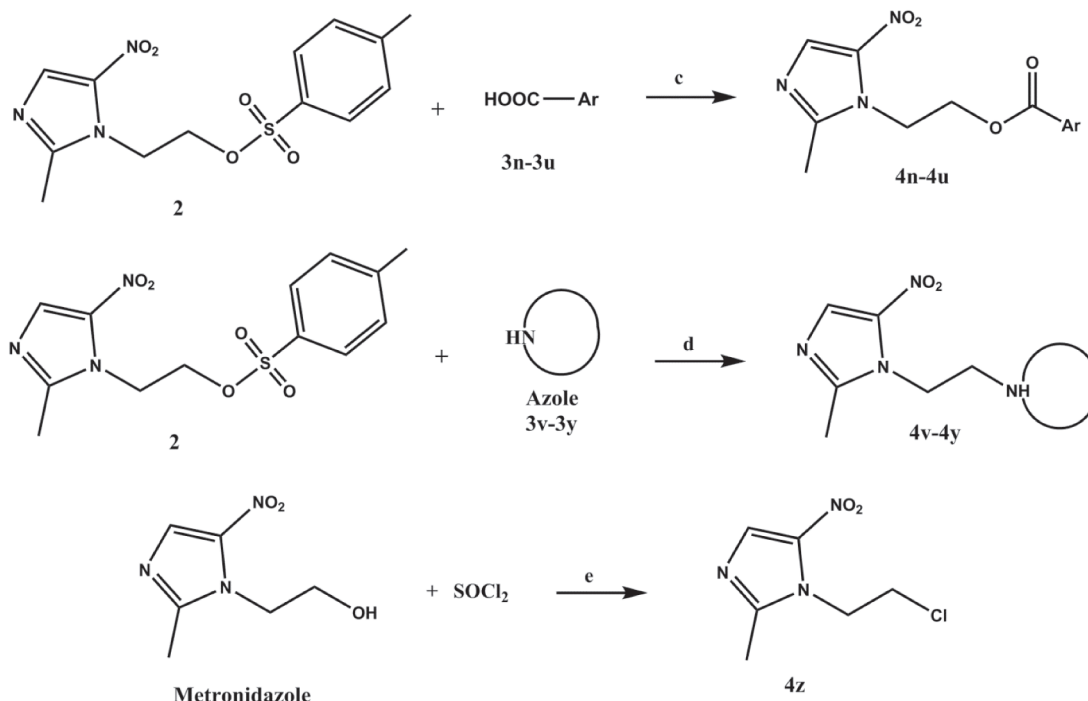
**MOTS** (2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethyl 4-methylbenzenesulfonate) was synthesized from metronidazole (MTZ) by a classical tosylation reaction which has been precedently reported by Wang et al.<sup>4</sup>. In this method, a mixture of p-Toluenesulfonyl chloride, metronidazole and pyridine was stirred in chloroform at 0 °C for 24 h to

provide the desired **MOTS** (yield: 74%). Then, **MOTS**, related phenol and potassium carbonate were heated (24 h) in DMF at 80 °C to form compounds 4a-4n. Whereas, for the formation of 4o-4x, related aromatic carboxylic acid was added and a longer duration (~72 h) of heating was applied. The last series (Azole derivatives, 4v-4y) were synthesized according to the route outline in scheme 2. The general condition was the same as for compounds 4a-4m, while the workup procedure was different. Compound 4z was synthesized as the literary method<sup>17</sup>. Chemical structures of the synthesized compounds are summarized in Tables 1 and 2.

### 2.2. Cell proliferation assay

The cytotoxicity of the compounds was evaluated on three cancer cell lines by MTT method. Table 3 shows IC<sub>50</sub> values of 26 newly synthesized aryloxy, carboxy, and azole derivatives of metronidazole on three cancer cell lines. Data are presented as mean ± S.E.M.

The results show that compound 4l had the most potent cytotoxic effect on HT-29 cell line (IC<sub>50</sub> value about 77 µg/mL). Compounds 4d and 4m also showed acceptable cytotoxicity on this cell line (IC<sub>50</sub> about



**Scheme 2.** Reagents and conditions: K<sub>2</sub>CO<sub>3</sub>, DMF, 80 °C, 72 h (c); K<sub>2</sub>CO<sub>3</sub>, DMF, 80 °C, 24 h (d) Compound 4z was synthesized as the literary method (e).<sup>17</sup>

Table 1

Structures of compounds **4a–4m**.

| 4a–4m      |    |
|------------|----|
| Compound   | Ar |
| <b>4a</b>  |    |
| <b>4b</b>  |    |
| <b>4c</b>  |    |
| <b>4d</b>  |    |
| <b>4e</b>  |    |
| <b>*4f</b> |    |
| <b>*4g</b> |    |
| <b>4h</b>  |    |
| <b>*4i</b> |    |
| <b>4j</b>  |    |
| <b>4k</b>  |    |
| <b>4l</b>  |    |
| <b>*4m</b> |    |

\* QSAR prediction set.

91 and 108  $\mu\text{g/mL}$ ). Compounds **4m** ( $\text{IC}_{50}$  about 96  $\mu\text{g/mL}$ ), **4l** ( $\text{IC}_{50}$  about 80  $\mu\text{g/mL}$ ), and **4d** ( $\text{IC}_{50}$  about 78  $\mu\text{g/mL}$ ) had the most powerful anti-proliferative effect on A549 cell line. Compounds, **4b**, **4i**, **4m**, **4d**, and **4l** were more cytotoxic for MCF7 cell line than other compounds.

With respect to the SAR, the following can be noted: 1- The presence of electron withdrawing groups ( $-\text{F}$   $-\text{Cl}$   $-\text{NO}_2$   $-\text{CN}$ ) at the para position on phenyl ring enhances activity (**4b**, **4h**, **4l**, **4k**). 2-Substitution at the meta position is also tolerated and does not drastically affect potency (**4g**, **4e**), whereas *ortho*-substitution is not tolerated and lead to dramatic loss of activity (**4c**, **4j**). 3-A more dramatic improvement in activity could be achieved by the presence of a proton donor group (e.g. OH) at the para position (comp. **4l**). 4-It seems that there is a correlation between the lipophilicity and reactivity since compound **4m** has a top ranked antiproliferative activity and compound **4d** has a greater potency than **4g**. 5-In the phenolic series, the more potent the electron withdrawing group is the more cytotoxic the compound will be (e.g. **4f** is more potent than **4e**).

### 2.3. Apoptosis assay

After determining the  $\text{IC}_{50}$  values of the compounds, three of them were chosen to be investigated by flow cytometry. The percent of necrotic, late apoptotic, viable and early apoptotic cells are seen in Q1, Q2, Q3, and Q4 parts respectively (Fig. 1). The results show that the compounds cytotoxicity is mostly via induction of apoptosis.

### 2.4. QSAR analysis

MLR model building was carried out on ten fingerprints output from the GA variable selection method. The statistical parameters of the best

constructed model are presented in Table 4.

The statistical parameters of the model show that it is of a high quality. The plot of predicted versus measured activity is shown in Fig. 2.

The dictionary based fingerprints, their SMARTS pattern, regression coefficients per each and molecules that had value one for each of them is shown in Table 5.

The intercept value of developed QSAR model is 2.930. Fingerprints chosen in the constructed model are KR 2695, CDK extended fingerprint (ExtFP) 607, 359, 935, 704, 161, and CDK 271, 161, 539, 600 and atom pairs 2D fingerprint (AD2D) 393. The most effective fingerprints in the biological activity based on regression coefficients are KR 2695, ExtFP 935, and CDK 271 with weight values  $-0.90$ ,  $0.692$ ,  $-0.507$  respectively. Fingerprints ExtFP and CDK are among the hashed fingerprints. Therefore, herein just fingerprints KR 2695 and AD2D 393 are interpretable. Fingerprint KR 2695 with coefficient value  $-0.90$  plays an important role in the structure activity relationship. KR2695 has an adverse effect on the biological activity: having this pattern leads to less biological activity. It appeared in compounds **4c**, **4j**, and **4t** with  $\text{pIC}_{50}$  values 2.872, 2.700, and 2.834 that are among the least active compounds of the activity list. In compounds **4c**, **4j**, and **4t**, oxygen attached to the phenyl ring is in the *ortho* ring position with another substituent, Cl, NO<sub>2</sub> and COC respectively. Another pattern for the biological activity is fingerprint AD2D 393 with coefficient value 0.122 which shows that its occurrence improves the biological activity. It occurred in compounds **4i**, **4j**, **4k**, **4n**, **4q**, **4s**, **4t**, **4u**, **4m**, **4o**, **4p**, and **4r** with  $\text{pIC}_{50}$  values 3.61, 2.7, 3.491, 3.051, 2.911, 2.853, 2.834, 3.108, 3.6601, 3.048, 3.116, and 3.053 respectively. Several of these compounds like **4m** are among the active compounds in the set. It is the presence of C–O at topological distance 6 and is suitable for the biological activity. This fingerprint along with ExtFP 359, and CDK 600 are the least important fingerprints effective in the biological activity with weights of  $-0.117$ , and  $-0.135$  respectively.

### 2.5. Molecular docking studies

Based on previous reports on anticancer activity of some metronidazole derivatives [8–12], molecular docking was performed to identify which anticancer target protein the synthesized compounds probably affect. The compounds were fitted into the active site of epidermal growth factor receptor (EGFR, PDB code: 1 M17), tubulin (PDB code: 1SAO) and focal adhesion kinase (FAK, PDB code: 2ETM). The results have been plotted and presented in Fig. 3.

It showed that the interaction energy between the synthesized compounds and the focal adhesion kinase is the lowest. The compound **4m** had the best energy of the ligand-receptor complex ( $\Delta G = -10.72$  Kcal/mol) with FAK (Fig. 3). Also, **4m** was the most potent compound in terms of  $\text{IC}_{50}$  (64  $\mu\text{M}$  on MCF7) in cytotoxicity evaluation. Afterwards, the binding modes of compounds **4m** and FAK were depicted in Fig. 4a and b.

In the binding mode, compound **4m** is strongly bound to the ATP binding site of FAK via multiple hydrophobic interactions and three hydrogen bonds as well as one  $\pi$ -cation interaction. The NH group of Gln432 formed one hydrogen bond (distance = 2.69 Å) with oxygen atom of NO<sub>2</sub> moiety of **4m**. Also, the NH group of Phe433 formed one hydrogen bond (distance = 2.88 Å) with the other oxygen atom of NO<sub>2</sub> moiety of **4m**. Meanwhile, another hydrogen bond was formed between the end group Lys454 and oxygen of naphthoxy group (distance = 2.66 Å) of **4m**. Also, the imidazole ring of **4m** formed a  $\pi$ -cation interaction with Lys454 (Fig. 4b).

### 2.6. Molecular dynamics studies

Potential energy was calculated and the MD simulation results demonstrated that the system was equilibrated during the first 30 ns. The results show that the potential energy decreases and finally takes

Table 2

Structures of compounds **4n–4y**.

| Compound   | Ar | Compound  | Ar |
|------------|----|-----------|----|
| <b>4n</b>  |    | <b>4u</b> |    |
| <b>*4o</b> |    | <b>4v</b> |    |
| <b>*4p</b> |    | <b>4w</b> |    |
| <b>4q</b>  |    | <b>4x</b> |    |
| <b>4r</b>  |    | <b>4y</b> |    |
| <b>*4s</b> |    |           |    |
| <b>4t</b>  |    |           |    |
|            |    |           |    |

\*QSAR prediction set.

constant value soon after starting the simulation (Fig. 5a).

The scope of structural changes of protein respect to the initial structure during the MD simulations was monitored by plotting the root mean square deviation (RMSD) of protein in Fig. 5b. The Fig. 5b shows the structural stability of 2ETM after about 12 ns from the start of MD simulations. For 2ETM, it is simply seen that the RMSD increases steadily up to 0.37 nm. Local 2ETM mobility was analyzed by calculating the time-averaged root mean square fluctuation (RMSF). The RMSF showed that molecular system was stable. It measured the deviation between the positions of the 2ETM and some reference position, which indicates the local changes in the 2ETM (Fig. 5c). The peaks show the areas of 2ETM fluctuating during the simulation. The radius of gyration (Rg) values of 2ETM and **4m**-bound-2ETM complex were determined and plotted as a function of time, as shown in Fig. 5d. In this system, initially, the Rg value of the **4m**-bound 2ETM was 0.4 nm then its value was stabilized at about 30 ns, indicating that the MD simulation achieved equilibrium after 12 ns.

### 3. Conclusions

The present study describes the synthesis, structure elucidations, and investigation of cytotoxic activities of a series of novel metronidazole aryloxy, carboxy andazole derivatives on three cancer cell lines evaluated by MTT assay. Also, QSAR, docking, and molecular dynamics simulation studies were carried out to examine important fragments and interactions responsible for biological activity. Among three investigated cancer targets, interaction of synthesized compounds

with FAK was the lowest so that compound **4m** showed the best interaction energy of the ligand-receptor complex with FAK. Also, **4m** was the most potent compound ( $IC_{50} = 64 \mu M$  on MCF7) in cytotoxicity assessment while having an acceptable apoptotic effect. So, it can be a potential anticancer candidate that may act through inhibition of FAK. Moreover, compound **4l** was the most potent cytotoxic one on HT-29 cell line ( $IC_{50}$  value about  $77 \mu g/mL$ ). Compounds **4m**, **4l**, and **4d** had the most powerful anti-proliferative effect on A549 cell line. Also, compounds, **4b**, **4i**, **4m**, **4d**, and **4l** were more cytotoxic for MCF7 cell line in comparison to other compounds. The results of apoptosis assay show that the cytotoxicity of these compounds is mostly via induction of apoptosis.

### 4. Experimental

#### 4.1. Materials and measurements

All chemicals and reagents used in current study were purchased from Merck and Sigma companies. TLC was performed on the Aluminium-backed silica gel sheets (silica GF254) and visualized in UV light (254 nm). Melting points (uncorrected) were determined on an Electrothermal-9100 apparatus. IR spectra were recorded on a Bruker FTIR (Alpha model). All the  $^1H$  NMR spectra were recorded on a Bruker AVANCE III 300 MHz model Spectrometer.  $^{13}C$  NMR spectra were recorded on the same instruments at 75 MHz, using TMS as an internal standard. Elemental analyses were performed using a Heraeus CHN-O-Rapid analyzer.

**Table 3**

Compounds IC<sub>50</sub> (μg/mL) for three different cell lines. Data are presented as mean ± S.E.M.

| Compound | Cell line, IC <sub>50</sub> (μg/mL) |                |                |
|----------|-------------------------------------|----------------|----------------|
|          | HT29                                | A549           | MCF7           |
| 4a       | 134.31 ± 3.96                       | 107.44 ± 3.25  | 106.43 ± 5.06  |
| 4b       | 102.32 ± 4.50                       | 129.06 ± 2.14  | 85.81 ± 5.06   |
| 4c       | 552.13 ± 14.01                      | 574.48 ± 16.39 | 378.06 ± 11.26 |
| 4d       | 108.82 ± 6.64                       | 77.90 ± 6.98   | 69.65 ± 8.51   |
| 4e       | 158.75 ± 8.26                       | 149.63 ± 9.07  | 187.64 ± 9.40  |
| 4f       | 140.389                             | 225.705        | 115.828        |
| 4g       | 198.99 ± 7.45                       | 121.85 ± 4.51  | 110.13 ± 3.18  |
| 4h       | 115.61 ± 11.71                      | 110.38 ± 13.48 | 126.63 ± 16.06 |
| 4i       | 111.81 ± 2.43                       | 108.36 ± 1.73  | 71.67 ± 2.90   |
| 4j       | 580.73 ± 18.14                      | 573.39 ± 15.49 | 582.73 ± 19.55 |
| 4k       | 104.04 ± 5.77                       | 144.30 ± 4.18  | 109.05 ± 6.45  |
| 4l       | 76.84 ± 2.44                        | 79.81 ± 3.05   | 83.18 ± 2.78   |
| 4m       | 94.82 ± 12.60                       | 96.06 ± 2.36   | 64.90 ± 12.57  |
| 4n       | 287.67                              | 280.90         | 244.65         |
| 4o       | 145.85                              | 169.60         | 262.36         |
| 4p       | 277.33                              | 194.01         | 271.38         |
| 4q       | 279.23                              | 254.89         | 380.46         |
| 4r       | 358.81                              | 183.97         | 255.80         |
| 4s       | 293.07                              | 281.17         | 428.09         |
| 4t       | 282.60                              | 289.08         | 447.14         |
| 4u       | 286.54                              | 167.32         | 206.60         |
| 4v       | 114.62 ± 8.57                       | 239.97 ± 7.38  | 461.97 ± 7.80  |
| 4w       | 142.03 ± 2.07                       | 195.46 ± 5.31  | 157.23 ± 4.65  |
| 4x       | 148.00 ± 4.80                       | 219.43 ± 6.33  | 103.16 ± 5.43  |
| 4y       | 537.97 ± 18.73                      | 701.25 ± 20.13 | 384.45 ± 14.35 |
| 4z       | 310.78                              | 300.49         | 480.11         |
| MOTS     | 327.50                              | 196.27         | 159.83         |
| MTZ      | 278.08                              | 223.78         | 347.96         |
| MTX      | 47.82 ± 2.50                        | 39.33 ± 1.96   | 31.48 ± 2.14   |

## 4.2. Chemistry

### 4.2.1. General procedure for synthesis of MOTS

A mixture of p-Toluenesulfonyl chloride (9.39 g, 50 mmol), metronidazole (8.55 g, 50 mmol) and pyridine (6 mL, 74.5 mmol) in chloroform (16 mL) was stirred at 0 °C in an ice bath. After being stirred for 24 h, the precipitate formed was filtered and washed with water and dried in air.

**4.2.1.1. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-methylbenzenesulfonate (MOTS).** White crystals, yield: 80%, m.p. 151–153 °C. IR (KBr, ν<sub>max</sub>/cm<sup>-1</sup>): 3034, 2975, 1595, 1526, 1366, 1175. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300 MHz) δppm: 7.93 (s, 1H, ArH), 7.62–7.59 (q, 3J = 3 Hz, 2H, ArH), 7.41–7.39 (d, 3J = 6 Hz, 2H, ArH), 4.56 (t, 3J = 6 Hz, 2H, OCH<sub>2</sub>), 4.40 (t, 3J = 6 Hz, 2H, NCH<sub>2</sub>), 2.41 (s, 6H, 2CH<sub>3</sub>). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 75 MHz) δppm: 152.02, 145.74, 138.82, 133.48, 133.90, 130.66, 127.73, 69.10, 45.10, 21.38, 14.43.

### 4.2.2. General procedure for the synthesis of 4a–4m

To a mixture of respected phenol (3a–3n, 10 mmol) and MOTS (3.24 g, 10 mmol) in DMF (10 mL) was added potassium carbonate (1.38 g, 10 mmol) and heated at 80 °C for 24 h. The progress of the reaction was monitored by TLC and ethanol/ethyl acetate was used as an eluent. After completion of the reaction the mixture was poured into ice water (100 mL). The resulting precipitate was filtered, washed with water and dried in air.

**4.2.2.1. 2-methyl-5-nitro-1-(2-phenoxyethyl)-1H-imidazole (4a).** Brown crystals, yield: 74%, m.p. 102–104 °C. IR (KBr, ν<sub>max</sub>/cm<sup>-1</sup>): 3035, 2925, 1596, 1534, 1366, 1189. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300 MHz) δppm: 8.04 (s, 1H, ArH), 7.30–6.37 (m, 5H, ArH), 4.71 (t, 3J = 6 Hz, 2H, OCH<sub>2</sub>), 4.32 (t, 3J = 3 Hz, 2H, NCH<sub>2</sub>), 2.53 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 75 MHz) δppm: 158.21, 152.31, 133.36, 130.03, 128.51,

125.97, 121.49, 114.76 66.63, 45.75, 14.62. For C<sub>12</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub>, calcd.: C, 58.29; H, 5.30; N, 16.99; Found: C, 58.85; H, 5.27; N, 16.43.

**4.2.2.2. 1-(2-(4-fluorophenoxy)ethyl)-2-methyl-5-nitro-1H-imidazole (4b).** Brown crystals, yield: 83%, m.p. 84–86 °C. IR (KBr, ν<sub>max</sub>/cm<sup>-1</sup>): 3021, 2937, 1580, 1526, 1365, 1144. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300 MHz) δppm: 8.03 (s, 1H, ArH), 7.10 (t, 3J = 3 Hz, 2H, ArH), 6.92–6.87 (m, 2H, ArH), 4.70 (t, 3J = 3 Hz, 2H, OCH<sub>2</sub>), 4.30 (t, 3J = 3 Hz, 2H, NCH<sub>2</sub>), 2.52 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 75 MHz) δppm: 155.66, 154.69, 152.27, 138.94, 133.33, 116.49, 116.05, 67.32, 45.71, 14.58. For C<sub>12</sub>H<sub>12</sub>FN<sub>3</sub>O<sub>3</sub>, calcd.: C, 54.34; H, 4.56; N, 15.84; Found: C, 54.05; H, 4.27; N, 15.43.

**4.2.2.3. 1-(2-(2-chlorophenoxy)ethyl)-2-methyl-5-nitro-1H-imidazole (4c).** Yellow crystals, yield: 77%, m.p. 129–131 °C. IR (KBr, ν<sub>max</sub>/cm<sup>-1</sup>): 3010, 2943, 1589, 1530, 1361, 1186. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300 MHz) δppm: 8.07 (s, 1H, ArH), 7.42–6.94 (m, 4H, ArH), 4.79 (t, 3J = 6 Hz, 2H, OCH<sub>2</sub>), 4.43 (t, 3J = 6 Hz, 2H, NCH<sub>2</sub>), 2.62 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 75 MHz) δppm: 154.30, 152.71, 139.13, 133.48, 130.61, 128.71, 122.74, 121.47, 114.52, 68.09, 45.68, 14.73. For C<sub>12</sub>H<sub>12</sub>ClN<sub>3</sub>O<sub>3</sub>, calcd.: C, 51.16; H, 4.29; N, 14.92; Found: C, 51.55; H, 4.17; N, 14.43.

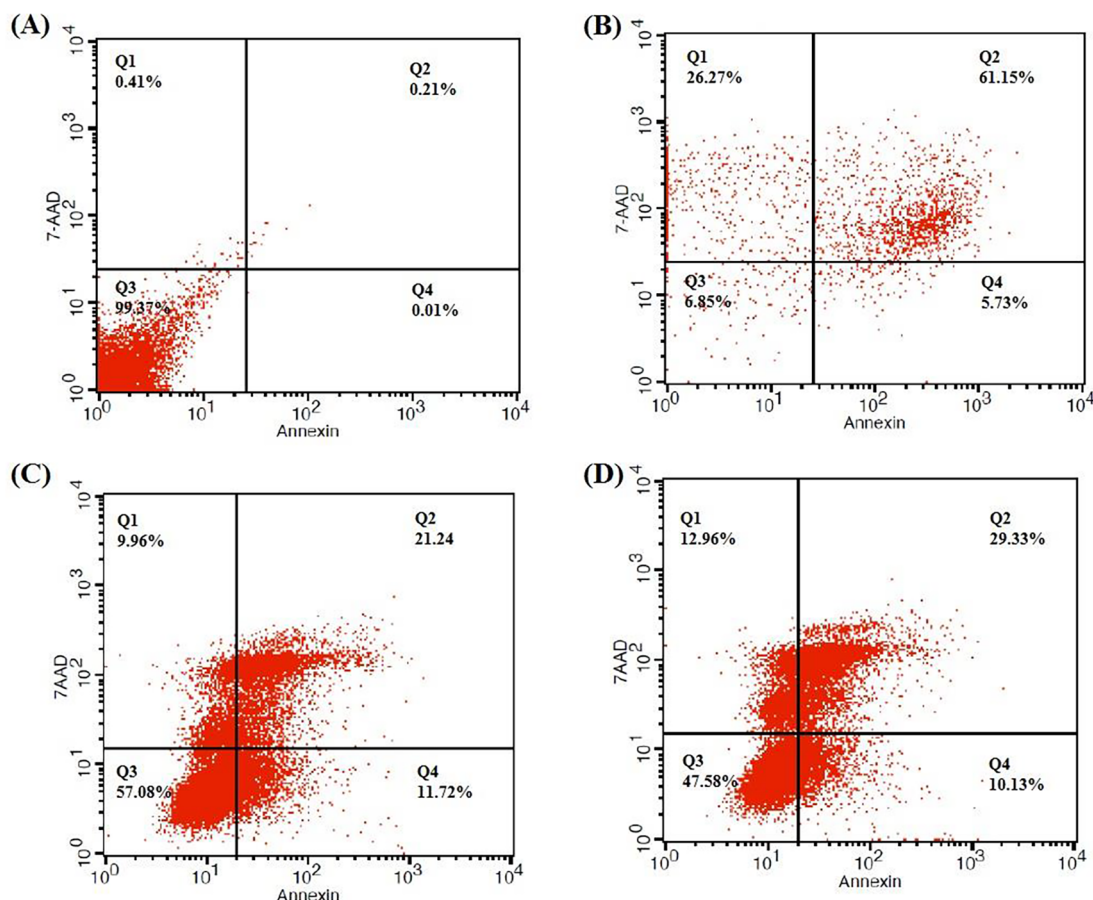
**4.2.2.4. 1-(2-(3,4-dichlorophenoxy)ethyl)-2-methyl-5-nitro-1H-imidazole (4d).** Brown crystals, yield: 68%, m.p. 147–150 °C. IR (KBr, ν<sub>max</sub>/cm<sup>-1</sup>): 3020, 2989, 1599, 1527, 1361, 1155. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300 MHz) δppm: 8.04 (s, 1H, ArH), 7.49 (d, 3J = 3 Hz, 1H, ArH), 7.24–7.21 (m, 1H, CH), 6.94–6.90 (m, 1H, ArH), 4.70 (t, 3J = 3 Hz, 2H, OCH<sub>2</sub>), 4.37 (t, 3J = 3 Hz, 2H, NCH<sub>2</sub>), 2.52 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 75 MHz) δppm: 157.62, 152.23, 133.35, 132.25, 132.10, 131.44, 123.25, 116.77, 115.96, 67.60, 45.73, 14.59. For C<sub>12</sub>H<sub>11</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>3</sub>, calcd.: C, 45.59; H, 3.51; N, 13.29; Found: C, 45.05; H, 3.29; N, 13.03.

**4.2.2.5. 1-(2-(4-chloro-3-methylphenoxy)ethyl)-2-methyl-5-nitro-1H-imidazole (4e).** Yellow crystals, yield: 73%, m.p. 107–109 °C. IR (KBr, ν<sub>max</sub>/cm<sup>-1</sup>): 3072, 2946, 1577, 1530, 1371, 1174. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300 MHz) δppm: 8.03 (s, 1H, ArH), 7.26 (d, 3J = 9 Hz, 1H, ArH), 6.90 (s, 1H, ArH), 6.74 (d, 3J = 3 Hz, 1H, ArH), 4.70 (t, 3J = 3 Hz, 2H, OCH<sub>2</sub>), 4.30 (t, 3J = 3 Hz, 2H, NCH<sub>2</sub>), 2.52 (s, 3H, CH<sub>3</sub>), 2.26 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 75 MHz) δppm: 156.99, 152.28, 138.95, 137.05, 133.36, 129.95, 125.49, 117.46, 113.99, 67.06, 45.68, 20.13, 14.60. For C<sub>13</sub>H<sub>14</sub>ClN<sub>3</sub>O<sub>3</sub>, calcd.: C, 52.80; H, 4.77; N, 14.21; Found: C, 53.05; H, 4.29; N, 14.03.

**4.2.2.6. 1-(2-(4-fluoro-3-methylphenoxy)ethyl)-2-methyl-5-nitro-1H-imidazole (4f).** Yellow crystals, yield: 70%, m.p. 95–97 °C. IR (KBr, ν<sub>max</sub>/cm<sup>-1</sup>): 3042, 2954, 1599, 1526, 1363, 1185. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300 MHz) δppm: 8.05 (s, 1H, ArH), 7.04 (t, 3J = 12 Hz, 1H, ArH), 6.82 (m, 1H, ArH), 6.72 (m, 1H, ArH), 4.72 (t, 3J = 3 Hz, 2H, OCH<sub>2</sub>), 4.29 (t, 3J = 3 Hz, 2H, NCH<sub>2</sub>), 3.34 (s, 3H, CH<sub>3</sub>), 2.53 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 75 MHz) δppm: 154.34, 152.32, 133.38, 125.84, 125.40, 117.43, 116.00, 115.68, 112.94, 67.51, 45.68, 14.73. For C<sub>13</sub>H<sub>14</sub>FN<sub>3</sub>O<sub>3</sub>, calcd.: C, 55.91; H, 5.05; N, 15.05; Found: C, 55.53; H, 5.19; N, 15.41.

**4.2.2.7. 1-(2-(3-chloro-4-fluorophenoxy)ethyl)-2-methyl-5-nitro-1H-imidazole (4g).** Yellow crystals, yield: 75%, m.p. 136–139 °C. IR (KBr, ν<sub>max</sub>/cm<sup>-1</sup>): 3041, 2946, 1594, 1528, 1363, 1187. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300 MHz) δppm: 8.04 (s, 1H, ArH), 7.31 (t, 3J = 3 Hz, 1H, ArH), 7.16 (m, 1H, ArH), 6.89 (m, 1H, ArH), 4.70 (t, 3J = 6 Hz, 2H, OCH<sub>2</sub>), 4.34 (t, 3J = 6 Hz, 2H, NCH<sub>2</sub>), 2.53 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 75 MHz) δppm: 154.84, 152.29, 138.95, 133.36, 120.10, 117.69, 117.52, 116.34, 115.56, 67.69, 45.59, 14.60. For C<sub>12</sub>H<sub>11</sub>ClFN<sub>3</sub>O<sub>3</sub>, calcd.: C, 48.09; H, 3.70; N, 14.02; Found: C, 48.57; H, 3.27; N, 14.41.

**4.2.2.8. 2-methyl-5-nitro-1-(2-(4-nitrophenoxy)ethyl)-1H-imidazole**



**Fig. 1.** Percentages of healthy, apoptotic (early and late), and necrotic A549 cells after 16 h treatment with none (A), **4d** (B), **4l** (C), and **4m** (D). The cells were treated with 90  $\mu\text{g/mL}$  of each compound and stained with PE annexin V and 7AAD and analyzed with flow cytometer.

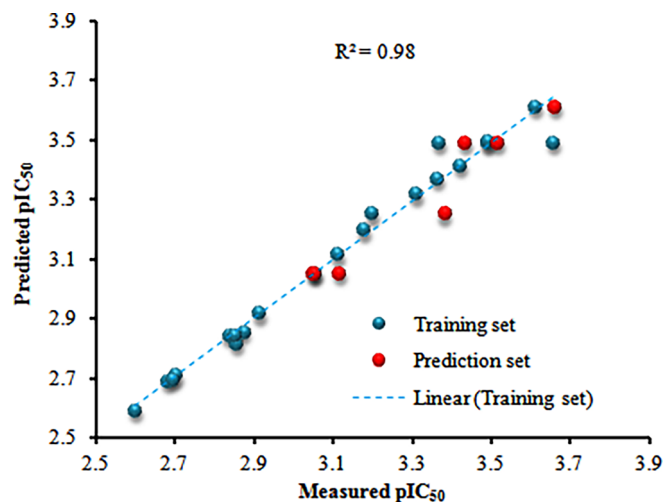
**Table 4**  
Statistical parameters of the best MLR model.

| $R^2_{\text{calibration}}$ | $R^2_{\text{cv}}^a$ | $R^2_{\text{pred}}^b$ | RMSEP <sup>c</sup> | F      | $R^2_{\text{randomization}}$ |
|----------------------------|---------------------|-----------------------|--------------------|--------|------------------------------|
| 0.978                      | 0.956               | 0.943                 | 0.063              | 44.054 | 0.558                        |

<sup>a</sup>  $R^2_{\text{cv}}$ :  $R^2$  cross validation.

<sup>b</sup>  $R^2_{\text{pred}}$ :  $R^2$  prediction.

<sup>c</sup> RMSEP: Root mean square error of prediction.



**Fig. 2.** The plot of predicted versus measured  $\text{pIC}_{50}$  of the QSAR model.

(**4h**). Brown crystals, yield: 75%, m.p. 170–173 °C. IR (KBr,  $\text{vmax/cm}^{-1}$ ): 3075, 2929, 1594, 1530, 1333, 1151.  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ , 300 MHz)  $\delta$ ppm: 8.20–8.17 (m, 2H, ArH), 8.03 (s, 1H, ArH), 7.13–7.10 (m, 2H, ArH), 4.76 (t,  $3J = 6$  Hz, 2H, OCH<sub>2</sub>), 4.50 (t,  $3J = 6$  Hz, 2H, NCH<sub>2</sub>), 2.54 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ , 75 MHz)  $\delta$ ppm: 163.55, 152.23, 141.72, 139.01, 133.36, 126.30, 115.51, 67.83, 45.47, 14.59. For  $\text{C}_{12}\text{H}_{12}\text{N}_4\text{O}_5$ , calcd.: C, 49.32; H, 4.14; N, 19.17; Found: C, 48.95; H, 4.29; N, 19.03.

#### 4.2.2.9. 2-methyl-5-nitro-1-(2-(3-nitrophenoxy)ethyl)-1H-imidazole

(**4i**). Brown crystals, yield: 80%, m.p. 144–147 °C. IR (KBr,  $\text{vmax/cm}^{-1}$ ): 3025, 2933, 1619, 1533, 1353, 1186.  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ , 300 MHz)  $\delta$ ppm: 8.03 (s, 1H, ArH), 7.83–7.36 (m, 4H, ArH), 4.75 (t,  $3J = 6$  Hz, 2H, OCH<sub>2</sub>), 4.48 (t,  $3J = 6$  Hz, 2H, NCH<sub>2</sub>), 2.55 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ , 75 MHz)  $\delta$ ppm: 158.77, 152.20, 149.15, 138.80, 133.22, 131.01, 122.36, 116.74, 109.29, 67.88, 45.49, 14.60. For  $\text{C}_{12}\text{H}_{12}\text{N}_4\text{O}_5$ , calcd.: C, 49.32; H, 4.14; N, 19.17; Found: C, 48.97; H, 4.27; N, 19.41.

#### 4.2.2.10. 2-methyl-5-nitro-1-(2-(2-nitrophenoxy)ethyl)-1H-imidazole

(**4j**). Brown crystals, yield: 71%, m.p. 84–87 °C. IR (KBr,  $\text{vmax/cm}^{-1}$ ): 3024, 2993, 1609, 1524, 1369, 1191.  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ , 300 MHz)  $\delta$ ppm: 8.05 (s, 1H, ArH), 7.83(d,  $3J = 3$  Hz, 1H, ArH), 7.63 (m, 1H, ArH), 7.35 (d,  $3J = 9$  Hz, 1H, ArH), 7.12 (m, 1H, ArH), 4.74 (t,  $3J = 6$  Hz, 2H, OCH<sub>2</sub>), 4.54 (t,  $3J = 6$  Hz, 2H, NCH<sub>2</sub>), 2.50 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ , 75 MHz)  $\delta$ ppm: 152.36, 150.91, 139.92, 138.84, 134.84, 133.56, 125.29, 121.55, 115.59, 68.37, 45.54, 14.33. For  $\text{C}_{12}\text{H}_{12}\text{N}_4\text{O}_5$ , calcd.: C, 49.32; H, 4.14; N, 19.17; Found: C, 49.56; H, 4.77; N, 18.96.

Table 5

Dictionary based fingerprints along with their SMARTS pattern, regression coefficients, and compounds that had value one for them.

| Rank | Fingerprint | SMARTS pattern                            | Regression coefficient | Molecules with bit value one                   |
|------|-------------|-------------------------------------------|------------------------|------------------------------------------------|
| 1    | KR2695      | [!#1]Oc1[cH][cH][cH][cH]c1[!#1]           | −0.90                  | 4c, 4j, 4t                                     |
| 2    | AD2D 393    | Presence of C–O at topological distance 6 | 0.122                  | 4i, 4j, 4k, 4n, 4q, 4s, 4t, 4u, 4m, 4o, 4p, 4r |

4.2.2.11. 1-(2-(2,4-dinitrophenoxy)ethyl)-2-methyl-5-nitro-1H-imidazole (**4k**). Brown crystals, yield: 72%, m.p. 184–187 °C. IR (KBr,  $\nu_{\max}/\text{cm}^{-1}$ ): 3051, 2985, 1607, 1530, 1347, 1187.  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz)  $\delta$ ppm: 8.51 (d,  $3J = 3\text{ Hz}$ , 1H, ArH), 8.48 (d,  $3J = 3\text{ Hz}$ , 1H, ArH), 8.06 (s, 1H, ArH), 7.61 (d,  $3J = 9\text{ Hz}$ , 1H, ArH), 4.79 (t,  $3J = 6\text{ Hz}$ , 2H, OCH<sub>2</sub>), 4.31 (t,  $3J = 6\text{ Hz}$ , 2H, NCH<sub>2</sub>), 2.51 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 75 MHz)  $\delta$ ppm: 156.17, 153.02, 140.41, 139.14, 138.52, 133.47, 129.99, 121.44, 116.41, 69.71, 45.68, 14.44. For  $\text{C}_{12}\text{H}_{11}\text{N}_5\text{O}_7$ , calcd.: C, 42.74; H, 3.29; N, 20.77; Found: C, 42.58; H, 3.17; N, 20.41.

4.2.2.12. 4-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethoxy)benzonitrile (**4l**). Yellow crystals, yield: 75%, m.p. 147–150 °C. IR (KBr,  $\nu_{\max}/\text{cm}^{-1}$ ): 3020, 2942, 2228, 1603, 1512, 1366, 1174.  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz)  $\delta$ ppm: 8.05 (s, 1H, ArH), 7.78 (d,  $3J = 9\text{ Hz}$ , 2H, ArH), 7.08 (d,  $3J = 9\text{ Hz}$ , 2H, ArH), 4.75 (t,  $3J = 6\text{ Hz}$ , 2H, OCH<sub>2</sub>), 4.46 (t,  $3J = 6\text{ Hz}$ , 2H, NCH<sub>2</sub>), 2.55 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 75 MHz)  $\delta$ ppm: 161.67, 152.26, 139.01, 134.70, 133.37, 126.06, 119.46, 116.00, 103.82, 67.32, 45.50, 14.61. For  $\text{C}_{13}\text{H}_{12}\text{N}_4\text{O}_3$ , calcd.: C, 57.35; H, 4.44; N, 20.58; Found: C, 57.55; H, 4.17; N, 20.43.

4.2.2.13. 2-methyl-1-(2-(naphthalen-2-yloxy)ethyl)-5-nitro-1H-imidazole (**4m**). Brown crystals, yield: 73%, m.p. 110–113 °C. IR (KBr,  $\nu_{\max}/\text{cm}^{-1}$ ): 3057, 2943, 1597, 1529, 1357, 1180.  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz)  $\delta$ ppm: 8.05 (s, 1H, ArH), 7.83–7.77 (m, 3H, ArH), 7.45 (t,  $3J = 6\text{ Hz}$ , 1H, ArH), 7.37–7.32 (m, 2H, ArH), 7.11–7.07 (m, 1H, ArH), 4.78 (t,  $3J = 6\text{ Hz}$ , 2H, OCH<sub>2</sub>), 4.46 (t,  $3J = 6\text{ Hz}$ , 2H, NCH<sub>2</sub>), 2.57 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 75 MHz)  $\delta$ ppm: 156.04, 152.37, 134.57,

133.39, 129.90, 129.09, 127.98, 127.17, 126.95, 124.26, 118.76, 107.34, 66.88, 45.65, 14.65.

#### 4.2.3. General procedure for the synthesis of **4n–4u**

To a mixture of respected aryl carboxylic acid (3o–3x, 10 mmol) and **MOTS** (3.24 g, 10 mmol) in DMF (10 mL) potassium carbonate was added (1.38 g, 10 mmol) and heated at 80 °C. After 3 days, the reaction mixture was poured into the ice water and the formed insoluble matter was recovered by filtration. The insoluble matter was dissolved in hot ethanol, whereupon the mixture was allowed to cool and crystals formed were collected and dried in air.

4.2.3.1. 4.2.3.1.2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl benzoate (**4n**). Yellow crystals, yield: 63%, m.p. 101–103 °C. IR (KBr,  $\nu_{\max}/\text{cm}^{-1}$ ): 3017, 2982, 1717, 1598, 1523, 1365, 1187.  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz)  $\delta$ ppm: 8.06 (s, 1H, ArH), 7.87–7.51 (m, 5H, ArH), 4.75 (t,  $3J = 6\text{ Hz}$ , 2H, OCH<sub>2</sub>), 4.65 (t,  $3J = 6\text{ Hz}$ , 2H, NCH<sub>2</sub>), 2.48 (s, 3H, CH<sub>3</sub>). For  $\text{C}_{13}\text{H}_{13}\text{N}_3\text{O}_4$ , calcd.: C, 56.72; H, 4.76; N, 15.27; Found: C, 57.06; H, 4.61; N, 15.56.

4.2.3.2. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 2-fluorobenzoate (**4o**). Brown crystals, yield: 75%, m.p. 90–92 °C. IR (KBr,  $\nu_{\max}/\text{cm}^{-1}$ ): 3024, 2987, 1721, 1612, 1525, 1355, 1185.  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz)  $\delta$ ppm: 8.05 (s, 1H, ArH), 8.01–7.31 (m, 4H, ArH), 4.73 (t,  $3J = 6\text{ Hz}$ , 2H, OCH<sub>2</sub>), 4.65 (t,  $3J = 6\text{ Hz}$ , 2H, NCH<sub>2</sub>), 2.46 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 75 MHz)  $\delta$ ppm: 163.60, 163.06, 152.00, 136.11, 135.99, 133.61, 132.11, 125.16, 117.75, 117.58, 64.10, 45.21,

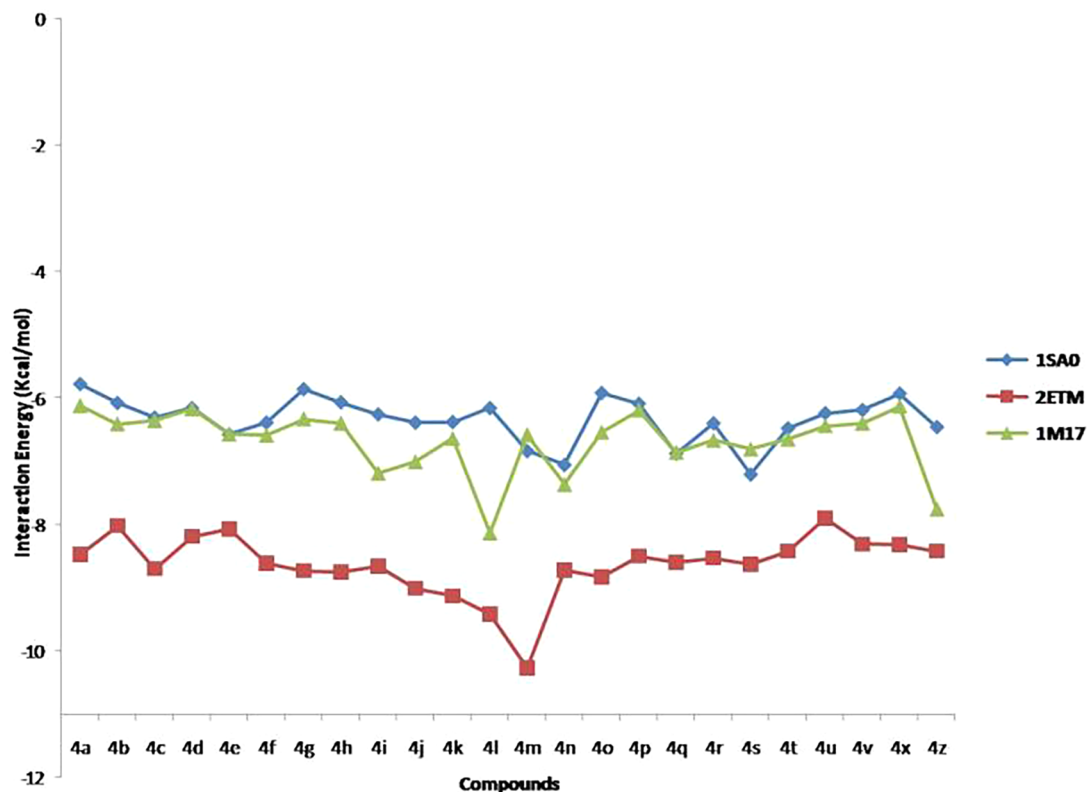
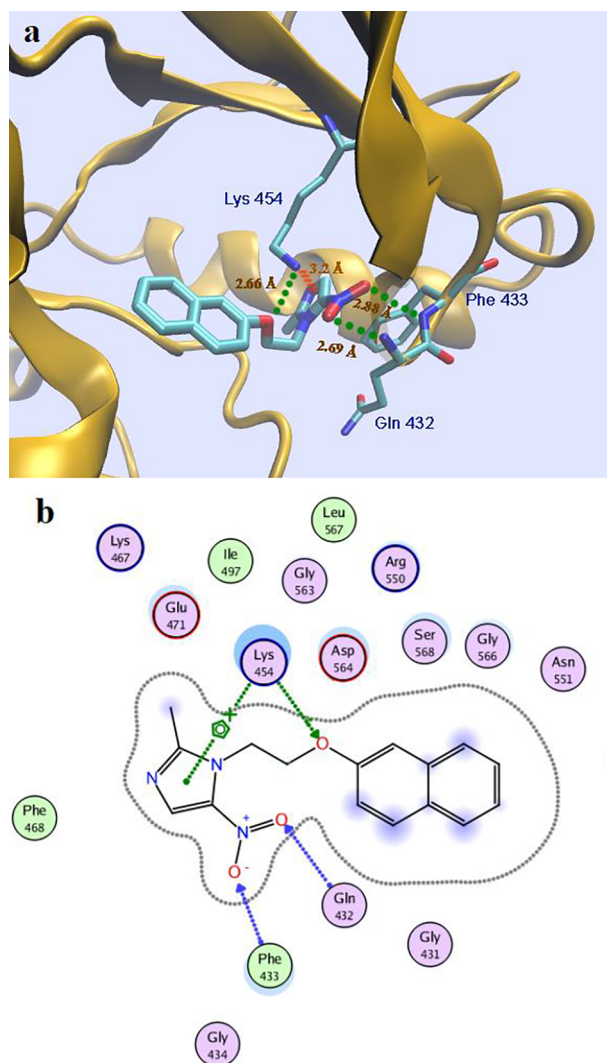


Fig. 3. Interaction energies of compounds **4a–4z** with EGFR (PDB code: 1M17), Tubulin (PDB code: 1SAO) and FAK (PDB code: 2ETM).



**Fig. 4.** Binding mode of compound **4m** with FAK. Only interacting residues were labeled. Hydrogen bonding interactions are shown in green dotted lines. This figure was made using VMD (a) and MOE (b).

14.31. For  $C_{13}H_{12}FN_3O_4$ , calcd.: C, 53.24; H, 4.12; N, 14.33; Found: C, 52.85; H, 4.57; N, 14.76.

**4.2.3.3. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-bromobenzoate (4p).** Yellow crystals, yield: 63%, m.p. 157–159 °C. IR (KBr,  $\nu_{\max}/\text{cm}^{-1}$ ): 3035, 2977, 1734, 1618, 1525, 1353, 1188.  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz)  $\delta$ ppm: 8.04 (s, 1H, ArH), 8.00–7.34 (m, 4H, ArH), 4.73 (t,  $3J = 3\text{ Hz}$ , 2H, OCH<sub>2</sub>), 4.65 (t,  $3J = 3\text{ Hz}$ , 2H, NCH<sub>2</sub>), 2.46 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 75 MHz)  $\delta$ ppm: 165.13, 151.90, 139.05, 133.65, 132.48, 131.41, 128.73, 128.17, 63.61, 45.13, 14.25. For  $C_{13}H_{12}BrN_3O_4$ , calcd.: C, 44.09; H, 3.42; N, 11.86; Found: C, 43.85; H, 3.53; N, 11.61.

**4.2.3.4. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 2-chlorobenzoate (4q).** Pale yellow crystals, yield: 73%, m.p. 101–103 °C. IR (KBr,  $\nu_{\max}/\text{cm}^{-1}$ ): 3021, 2959, 1733, 1612, 1525, 1365, 1184.  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz)  $\delta$ ppm: 8.07 (s, 1H, ArH), 7.70–7.44 (m, 4H, ArH), 4.74 (t,  $3J = 6\text{ Hz}$ , 2H, OCH<sub>2</sub>), 4.65 (t,  $3J = 6\text{ Hz}$ , 2H, NCH<sub>2</sub>), 2.45 (s, 3H, CH<sub>3</sub>). For  $C_{13}H_{12}ClN_3O_4$ , calcd.: C, 50.42; H, 3.91; N, 13.57; Found: C, 50.06; H, 3.63; N, 13.76.

**4.2.3.5. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-methylbenzoate (4r).** Brown crystals, yield: 71%, m.p. 105–108 °C. IR (KBr,  $\nu_{\max}/$

$\text{cm}^{-1}$ ): 3001, 2962, 1718, 1608, 1523, 1359, 1179.  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz)  $\delta$ ppm: 8.05 (s, 1H, ArH), 7.73 (d,  $3J = 6\text{ Hz}$ , 2H, ArH), 7.32 (m,  $3J = 6\text{ Hz}$ , 2H, ArH), 4.73 (t,  $3J = 3\text{ Hz}$ , 2H, OCH<sub>2</sub>), 4.62 (t,  $3J = 3\text{ Hz}$ , 2H, NCH<sub>2</sub>), 2.45 (s, 3H, CH<sub>3</sub>), 2.36 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 75 MHz)  $\delta$ ppm: 165.75, 151.87, 144.48, 139.04, 133.50, 129.86, 129.51, 126.76, 63.21, 45.13, 21.41, 14.33. For  $C_{14}H_{15}N_3O_4$ , calcd.: C, 58.13; H, 5.23; N, 14.53; Found: C, 58.45; H, 5.57; N, 14.81.

**4.2.3.6. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 3-methoxybenzoate (4s).** Yellow crystals, yield: 67%, m.p. 112–114 °C. IR (KBr,  $\nu_{\max}/\text{cm}^{-1}$ ): 3028, 2993, 1718, 1531, 1363, 1182.  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz)  $\delta$ ppm: 8.04 (s, 1H, ArH), 7.74 (d,  $3J = 6\text{ Hz}$ , 2H, ArH), 7.42 (d,  $3J = 6\text{ Hz}$ , 2H, ArH), 4.74 (t,  $3J = 6\text{ Hz}$ , 2H, OCH<sub>2</sub>), 4.62 (t,  $3J = 6\text{ Hz}$ , 2H, NCH<sub>2</sub>), 2.44 (s, 3H, OCH<sub>3</sub>), 2.35 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 75 MHz)  $\delta$ ppm: 165.83, 151.89, 139.08, 138.72, 134.65, 133.56, 129.91, 129.47, 129.18, 126.39, 63.21, 44.84, 21.39, 14.28. For  $C_{14}H_{15}N_3O_5$ , calcd.: C, 55.08; H, 4.95; N, 13.76; Found: C, 55.38; H, 4.65; N, 13.47.

**4.2.3.7. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 2-methoxybenzoate (4t).** Yellow crystals, yield: 65%, m.p. 50–53 °C. IR (KBr,  $\nu_{\max}/\text{cm}^{-1}$ ): 3010, 2979, 1708, 1602, 1523, 1365, 1184.  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz)  $\delta$ ppm: 8.06 (s, 1H, ArH), 7.56–6.97 (m, 4H, ArH), 4.70 (t,  $3J = 6\text{ Hz}$ , 2H, OCH<sub>2</sub>), 4.57 (t,  $3J = 6\text{ Hz}$ , 2H, NCH<sub>2</sub>), 3.75 (s, 3H, OCH<sub>3</sub>), 2.45 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 75 MHz)  $\delta$ ppm: 165.91, 158.70, 152.20, 138.50, 134.39, 133.56, 131.27, 120.48, 119.24, 112.96, 63.51, 56.05, 44.84, 14.31. For  $C_{14}H_{15}N_3O_5$ , calcd.: C, 55.08; H, 4.95; N, 13.76; Found: C, 55.35; H, 4.55; N, 13.51.

**4.2.3.8. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 2-fluorobenzoate (4u).** Brown crystals, yield: 65%, m.p. 108–109 °C. IR (KBr,  $\nu_{\max}/\text{cm}^{-1}$ ): 3018, 2998, 1714, 1612, 1530, 1363, 1177.  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz)  $\delta$ ppm: 8.04 (s, 1H, ArH), 7.98 (s, 1H, ArH), 7.22 (s, 1H, ArH), 6.70 (s, 1H, ArH), 4.70 (t,  $3J = 6\text{ Hz}$ , 2H, OCH<sub>2</sub>), 4.60 (t,  $3J = 6\text{ Hz}$ , 2H, NCH<sub>2</sub>), 2.49 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 75 MHz)  $\delta$ ppm: 157.47, 151.91, 148.49, 143.45, 138.79, 133.53, 119.20, 112.89, 63.17, 45.19, 14.13. For  $C_{11}H_{11}N_3O_5$ , calcd.: C, 49.81; H, 4.18; N, 15.84; Found: C, 50.18; H, 4.55; N, 15.47.

#### 4.2.4. General procedure for synthesis of 4v–4y

To a mixture of respected azole (3ab–3ae, 0.68 g, 10 mmol) and MOTs (3.24 g, 10 mmol) in DMF (10 mL) potassium carbonate was added (2.07 g, 15 mmol) and heated at 80 °C for 24 h. The progress of the reaction was monitored by TLC and hexane/ethyl acetate was used as an eluent. After completion, the reaction was cooled and diluted with 20 mL of ethyl acetate. The resulting salt was filtered off and organic layer remains at room temperature to stand. After 24 h, the precipitate formed was collected and recrystallized from acetone.

**4.2.4.1. 1-(2-(1H-imidazol-1-yl)ethyl)-2-methyl-5-nitro-1H-imidazole (4v).** White crystals, yield: 72%, m.p. 176 °C (dec). IR (KBr,  $\nu_{\max}/\text{cm}^{-1}$ ):  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz)  $\delta$ ppm: 8.05 (s, 1H, ArH), 7.44 (s, 1H, ArH), 7.03 (d,  $3J = 3\text{ Hz}$ , 1H, ArH), 6.87 (d,  $3J = 3\text{ Hz}$ , 1H, ArH), 4.58 (t,  $3J = 6\text{ Hz}$ , 2H, CH<sub>2</sub>), 4.42 (t,  $3J = 6\text{ Hz}$ , 2H, CH<sub>2</sub>), 1.79 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 75 MHz)  $\delta$ ppm: 151.86, 138.75, 138.02, 133.69, 129.25, 120.23, 47.65, 46.00, 13.07. For  $C_9H_{11}N_5O_2$ , calcd.: C, 48.86; H, 5.01; N, 31.66; Found: C, 48.46; H, 5.27; N, 31.21.

**4.2.4.2. 2-methyl-1-(2-(2-methyl-1H-imidazol-1-yl)ethyl)-5-nitro-1H-imidazole (4w).** Brown crystals, yield: 65%, m.p. 215 °C (dec). IR (KBr,  $\nu_{\max}/\text{cm}^{-1}$ ): 3013, 2956, 1534, 1366.  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz)  $\delta$ ppm: 8.06 (s, 1H, ArH), 6.82 (d,  $3J = 3\text{ Hz}$ , 1H, ArH), 6.69 (d,  $3J = 3\text{ Hz}$ , 1H, ArH), 4.56 (t,  $3J = 6\text{ Hz}$ , 2H, CH<sub>2</sub>), 4.33 (t,  $3J = 6\text{ Hz}$ , 2H, CH<sub>2</sub>), 2.08 (s, 3H, CH<sub>3</sub>), 1.85 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 75 MHz)  $\delta$ ppm: 151.96, 144.29, 139.38, 133.78, 127.26, 120.75, 47.04, 45.42, 13.26, 12.36. For  $C_{10}H_{13}N_5O_2$ , calcd.: C, 51.06; H, 5.57; N,

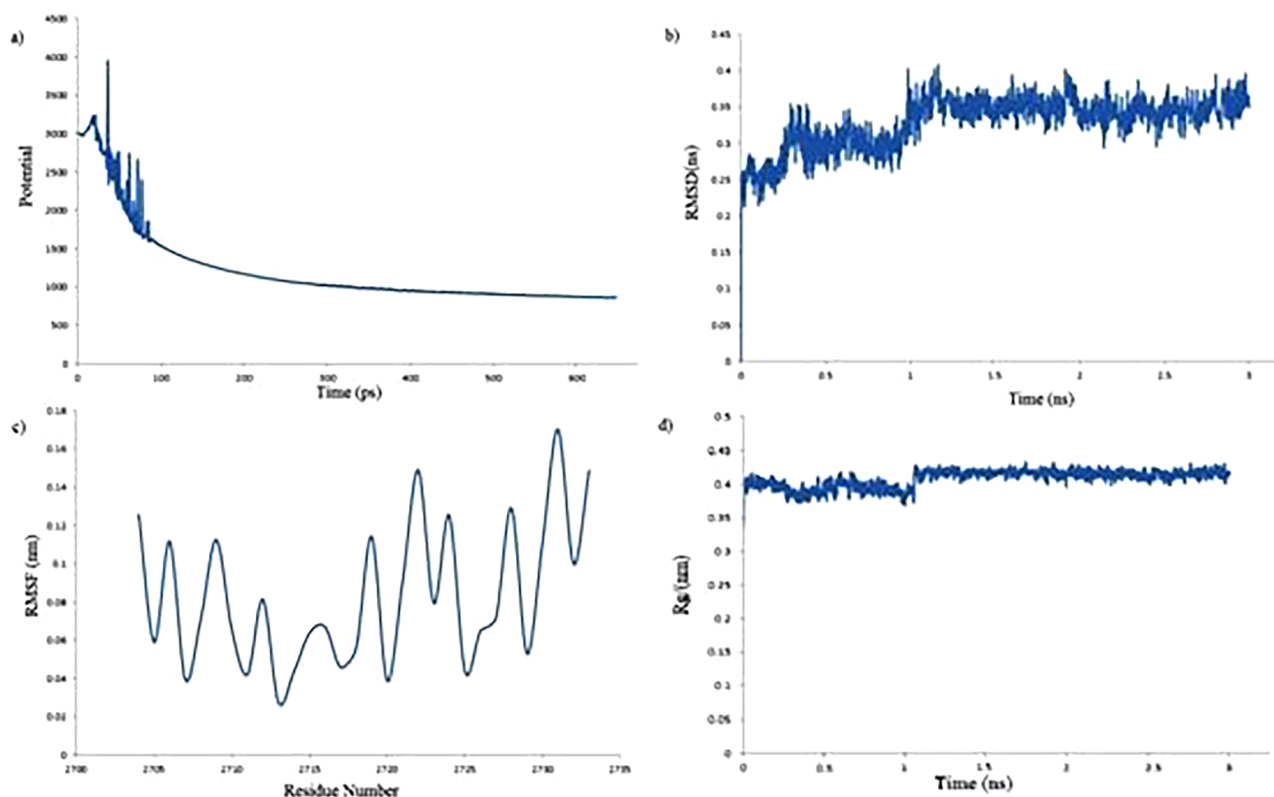


Fig. 5. Potential energy is shown as a function of time for 2ETM protein (a) root mean square deviation (RMSD) is shown as a function of time for 2ETM protein during 30 ns (b), root mean square fluctuation (RMSF) of the C $\alpha$  atoms in 2ETM plotted against time from MD simulation (c) and time evolution of the radius of gyration (Rg) during 30 ns MD simulation of ETM and **4m** (d).

29.77; Found: C, 51.46; H, 5.32; N, 30.13.

**4.2.4.3. 1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-1H-benzo[d]imidazole (**4x**).** Pale yellow crystals, yield: 68%, m.p. 108–109 °C. IR (KBr,  $\nu_{\text{max}}$ /cm<sup>-1</sup>): 3020, 2951, 1614, 1523, 1365. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300 MHz)  $\delta$ ppm: 8.03 (s, 1H, ArH), 8.01 (s, 1H, ArH), 7.67–7.18 (m, 4H, ArH), 4.73 (t, 3J = 3 Hz, 2H, OCH<sub>2</sub>), 4.68 (t, 3J = 3 Hz, 2H, NCH<sub>2</sub>), 1.64 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 75 MHz)  $\delta$ ppm: 151.49, 144.54, 143.76, 138.86, 134.05, 133.67, 123.22, 122.21, 120.03, 110.21, 46.32, 43.96, 13.17. For C<sub>13</sub>H<sub>13</sub>N<sub>5</sub>O<sub>2</sub>, calcd.: C, 57.56; H, 4.83; N, 25.82; Found: C, 57.18; H, 4.52; N, 25.56.

**4.2.4.4. 1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-1H-benzo[d][1,2,3]triazole (**4y**).** Yellow crystals, yield: 66%, m.p. 158–159 °C. IR (KBr,  $\nu_{\text{max}}$ /cm<sup>-1</sup>): 3020, 2951, 1616, 1590, 1365. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300 MHz)  $\delta$ ppm: 8.05–7.38 (m, 5H, ArH), 5.20 (t, 3J = 6 Hz, 2H, CH<sub>2</sub>), 4.82 (t, 3J = 6 Hz, 2H, CH<sub>2</sub>), 1.79 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 75 MHz)  $\delta$ ppm: 151.47, 145.39, 138.81, 133.75, 133.43, 128.11, 124.84, 119.65, 110.34, 47.22, 46.04, 13.04. For C<sub>12</sub>H<sub>12</sub>N<sub>6</sub>O<sub>2</sub>, calcd.: C, 52.94; H, 4.44; N, 30.87; Found: C, 52.66; H, 4.63; N, 30.66.

### 4.3. Cell proliferation assay

The cancer cell lines A549 (lung), MCF-7 (breast), and HT-29 (colorectal) were purchased from the Iranian Biological Resource Center (IBRC, Tehran, Iran). The cells were grown in DMEM culture media supplemented with 10% FBS (Gibco) and antibiotics (penicillin 100 U/mL and streptomycin 100  $\mu$ g/mL) at 37 °C in a CO<sub>2</sub> incubator (5% CO<sub>2</sub> and 95% relative humidity). After the cells reached the exponential growth,  $1 \times 10^4$  (for A549 and MCF-7) and  $1.2 \times 10^4$  (for HT-29) cells were seeded in each well of a flat-bottom microplate and incubated for 24 h at the above mentioned conditions. The newly synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) and the desired

concentrations of each compound ranging from 0.1 to 1000  $\mu$ g/mL were prepared by dilution in DMEM (without FBS). The amount of DMSO was less than 0.5% for the highest concentration. Each concentration was added to 3 wells and the microtiter plate was further incubated for 24 h. Methotrexate (MTX) was used as the reference drug. Another well was considered as the solvent control. To evaluate the effect of the compounds on cell growth and proliferation, 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) powder (Sigma, St. Louis, Mo.) dissolved in PBS (pH 7.2) to a concentration of 5 mg/mL was added to each well and the plates were incubated for 3–4 h at 37 °C in dark. Then, 100  $\mu$ L DMSO was added to each well and cell viability was determined by measuring the absorbance at 570 nm. The test was done 3 times for each compound and IC<sub>50</sub> values were calculated.

### 4.4. Apoptosis assay

Detection of apoptosis in cells was carried out by flow cytometry using PI annexin V apoptosis detection kit I (BD Pharmingen). A549 cells were treated with compounds **4d**, **4l**, and **4m** (90  $\mu$ g/mL) for 16 h, then trypsinized and washed twice in ice-cold PBS. The washed cells were suspended in binding buffer to a concentration of  $1 \times 10^6$  cells/mL. Then, the cells were stained with PE annexin V and 7-amino-actinomycin-D (7-AAD) based on manufacturer's protocol and incubated in dark. The measurement was done by FACS using flow cytometer (BD Biosciences, San Jose, CA, USA).

### 4.5. Quantitative structure activity relationship (QSAR)

28 compounds were used for QSAR model building. IC<sub>50</sub> values in  $\mu$ g/mL were taken in molar range and were expressed in negative logarithmic units, pIC<sub>50</sub> (-logIC<sub>50</sub>). The test set of 7 molecules was chosen based on Kennard-Stone algorithm<sup>18</sup>, and was used to determine the external predictive ability of the resulting QSAR model. The

remaining 21 compounds were treated as the training set and used to derive the regression model. Molecular fingerprints were utilized as molecular descriptors. Most fingerprints encode structural information in a binary bit string and are quickly calculable from the 2D-structure without any need to get the 3D structure of compounds or carry out the energy minimization. A set of 10,066 descriptors from 8 types of fingerprints including CDK (1024 bit standard hashed), CDK extended fingerprint (1024 keys), CDK graph only fingerprint (1024 keys), MACCS (166 keys), Pubchem (881 keys), FP4 (307 keys), atom pairs 2D fingerprints (780 keys) and Klekota-Roth fingerprints (4860 keys) were calculated using PaDEL-Descriptor software<sup>19</sup>. Except for CDK, CDK extended fingerprint, and CDK graph only fingerprint, all the others were among dictionary based fingerprints in which SMARTS patterns define functional groups.

Genetic algorithm (GA) as a highly efficient optimization algorithm was used to search the large dimensional space of molecular features that are responsible for the biological activities of these compounds<sup>20,21</sup>. GA is based on the principle of evolution. The selected fingerprints were introduced to MLR analysis. As, fingerprint descriptors consist of bit strings, just zero and one, ten bits were finally selected for model building while all compounds have had at least one bit on. The Genetic algorithm from QSAR-Tools ([http://teqip.jdvu.ac.in/QSAR\\_Tools/](http://teqip.jdvu.ac.in/QSAR_Tools/)) was used to apply GA for data generated from PaDEL-Descriptor<sup>22,23</sup>. A data treatment of removing variables with variance cut-off value of 0.001 and inter correlation cut-off value of 0.97 was carried out before running the variable selection. Default parameters of the GA program containing initial population = 100, total number of generations = 100, cross over probability = 1, mutation probability = 0.5, and number of best selected equations = 20 were used to set up the GA process while the run was repeated for at least 15 times and the best set of descriptors based on  $R^2$  and  $R^2_v$  was finally chosen for the model building. Chemoface software<sup>24</sup> then was used to build the MLR model, make predictions and getting statistical parameters.

#### 4.6. Docking simulations

Molecular docking of the synthesized compounds into the three dimensional X-ray structure of protein, was performed using the program AutoDock 4.2 (ADT)<sup>25</sup>. The three-dimensional structures of the above-mentioned compounds were built and energy minimized using the Openbabel 2.3.2 software<sup>26</sup>. The crystal structures of the protein complex were obtained from the RCSB Protein Data Bank (<http://www.rcsb.org>). All bound water molecules and ligands were removed from the protein and polar hydrogens were added to the proteins. Gasteiger Huckel charges were used for the ligand while Kollman charges were calculated for the protein. Molecular docking of all compounds was then carried out using the AutoDock 4.2 software. The grid box was focused, based on the cognate ligand with a spacing of 0.375 Å and 60 × 60 × 60 dimensions. Parameters of genetic algorithm were set to one hundred runs, 2,500,000 energy evaluations and 150 population size. Cluster analysis was done on the docked results, using an RMSD tolerance of 2 Å<sup>27</sup>.

#### 4.7. Molecular dynamics simulation

The molecular dynamics (MD) simulation has emerged as a modern tool to specify the interactions between proteins and drugs. The 3D structure of the protein was obtained from protein data bank (PDB) with PDB code of 2ETM for Adhesion Kinase with 4m inside of it. MD simulation was conducted for modeled system in explicit solvent using the GROMACS 4.5.3 package (<http://www.gromacs.org>). The model was solvated by 23,854 water molecules in a water box having edges at a distance of 0.7 nm. The reference temperature for coupling was 300 K and 1 atm pressure was retained. The final MD was carried out for 30,000 ps (30 ns).

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bmc.2018.12.003>.

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