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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis and molecular docking of novel structural hybrids of substituted isatin based pyrazoline and thiadiazoline as antitumor agents

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Abstract Cancer, which is considered to be the world's most serious illness cause 8.2 million deaths and this rate may double by 2030. We herein report a new series of 3-(2-(p-substituted)-2-((5-phenyl-1,3,4-thiadiazol-2-yl)imino)-2-(p-substituted)ethylidene)indolin-2-one **(15–19)** and 5-substituted-5′-substituted phenyl-2′,4′-dihydrospiro[indoline-3,3′-pyrazol]-2-one derivatives **(20–24)** as potent anticancer agents. These compounds were evaluated for in vitro antitumor activity against the National Cancer Institute panel of 60 cancer cell lines. Among all the synthesized compounds, two compounds **15** and **16** showed remarkable antitumor activity with GI_{50} (MG-MID) values of 0.65 & 0.72 µM, respectively against Non-small cell lung cancer. To gain insight for mode of binding with Epidermal Growth Factor Receptor kinase enzyme, these compounds were further subjected to docking studies.

Keywords EGFR kinase enzyme · Non-small cell lung cancer · Molecular docking · Antitumor activity · NCI

Introduction

Cancer, a life-threatening disease is the second common cause of death after cardiovascular diseases (Jemal et al. 2011). The American Chemical Society defines cancer as a group of diseases characterized by uncontrolled growth, and the spread of abnormal cells that left untreated may lead to death (Garcia et al. 2007). According to WHO global cancer report 2014, it is expected to increase 57% worldwide in next 20 years (Vineis and Wild 2014). By 2030, it is projected that there will be ~26 million new cancer cases and 17 million cancer deaths per year (Thun et al. 2010; Boyle and Levin 2008). The projected increase will be driven largely by growth and aging of populations and will be largest in low-resource and medium-resource countries (Abraham 2014).

Isatins are endogenous molecules present in human and mammals which exhibits diverse pharmacological profiles especially antimicrobial (Thadhaney et al. 2010; Bari et al. 2015), antitumor (Havrylyuk et al. 2012; Liang et al. 2014; Havrylyuk et al. 2015; Thi Lan Huong et al. 2015; Lesyk et al. 2015), antiviral (Varma and Khan 2014), anti-inflammatory (Socca et al. 2014), and antioxidant activities (Dandia et al. 2014). The isatin moiety present in wide range of compounds can act as inhibitors of apoptosis (Medvedev et al. 2007) targeting proteases (Zhou et al. 2006), caspases (Chapman et al. 2002) kinases (Cao et al. 2009) extracellular signal regulated protein kinase (ERK) (Cane et al. 2000). Isatin hybrids possessing heterocyclic analogues have been identified as potential antitumor agents and the most promising hybrid molecules are shown in Fig. 1 (Ibrahim et al. 2015; Eldehna et al. 2015; Ribeiro et al. 2016; Monteiro et al. 2014; Fares et al. 2015).

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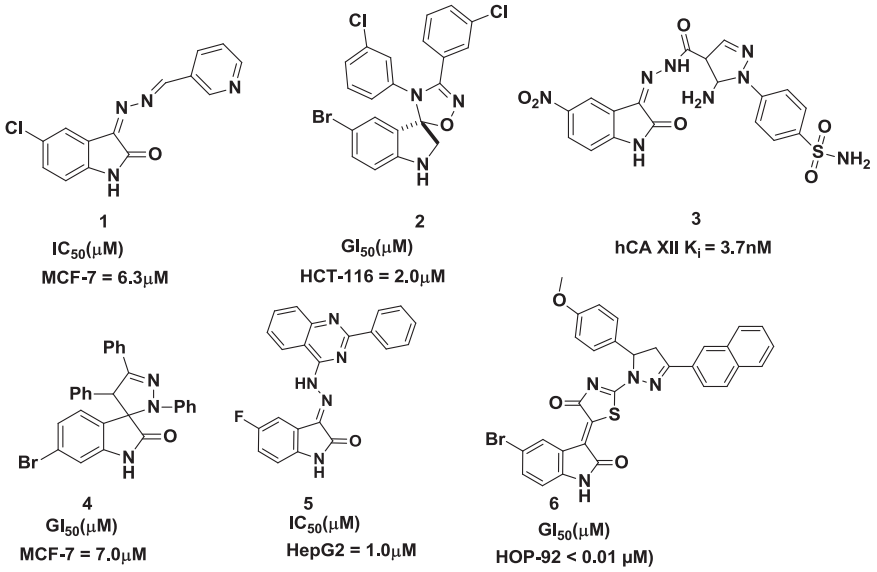


Fig. 1 Anticancer isatin hybrid molecules

In recent days, chemists have gained considerable attention on five membered aromatic systems having three heteroatoms at the symmetrical positions (i.e., pyrazolines, and thiadiazolines) due to their interesting biological activities (Yusuf and Jain 2014; Shih et al. 2015; Altıntop et al. 2015). On the other hand, various thiadiazole (Bursavich et al. 2007; Nikalje et al. 2015) and pyrazoline derivatives (Havrylyuk et al. 2012; Karthikeyan et al. 2015) as a potential chemotherapeutic agents. Spirooxindole were reported as an important class of heterocyclic scaffolds with promising anticancer activity (Reddy et al. 2015; Ziarani et al. 2016). Pyrazoline derivatives, are nitrogen heterocyclic compounds with electron rich property (Schmidt and Dreger 2011), widely occur in nature in the form of alkaloids (Shaaban et al. 2012), vitamins, pigments, and as constituents of plant and animal cell (Singh et al. 2009; Fall et al. 2002). Thiadiazole acts as "hydrogen binding domain" and "two electron donor system" with a constrained pharmacophore, has structural frameworks similar to several naturally occurring alkaloids that show a wide range of pharmaceutical and industrial importance (Chen et al. 2012).

In the present study, we herein report the synthesis of 3-(2-(p-substituted)-2-((5-phenyl-1,3,4-thiadiazol-2-yl) imino)-2-(p-substituted)ethylidene)indolin-2-one (**15–19**) and 5-substituted-5′-substituted phenyl-2′,4′-dihydrospiro [indoline-3,3′-pyrazol]-2-one derivatives (**20–24**) (Fig. 2). The new compounds were screened for their in vitro antitumor activity using the NCI's disease-oriented human cell lines assay. Docking simulations were performed using the X-ray crystallographic structure of the EGFR in complex with an inhibitor to explore the binding modes of these compounds at the active site.

Experimental

General methods

Melting points (°C) were determined on Toshniwal melting point apparatus and are uncorrected. Elemental analyses were performed on a Perkin-Elmer 240 elemental analyzer. Reactions were monitored using thin layer chromatography (TLC) on aluminum-backed precoated silica gel 60 F254 plates (E Merck). The FT-IR spectra of the compounds were recorded on thermo Nicolet Nexus 670S series.^{1}H NMR and ^{13}C NMR were recorded on a Avance-300 MHz instrument using TMS as an internal standard (chemical shifts in δ, ppm). Mass spectra were recorded on LC-MSD-Trap-SL using ESI(+) method. Column chromatography was performed by using Qualigen's silica gel for column chromatography (60–120 mesh). All the fine chemicals and reagents used were purchased from Sigma-Aldrich (St. Louis, USA).

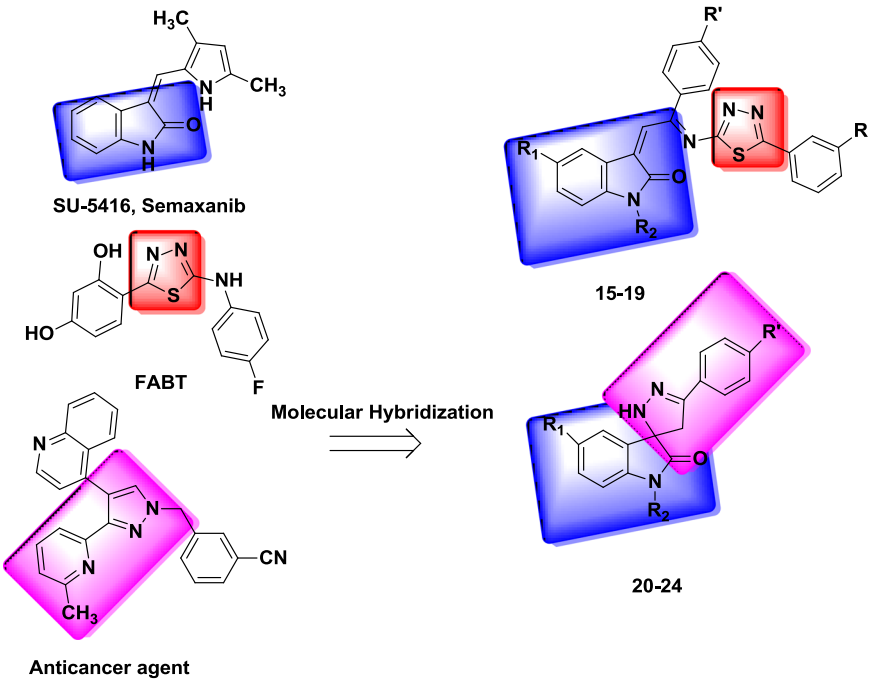
Synthesis of 2-amino-5-aryl-1,3,4-thiadiazole (1–3)

Compounds **1–3** were synthesized from substituted benzaldehyde and thiosemicarbazide and subsequently cyclized with bromine in glacial acetic acid. The solids were obtained and the residue was recrystallized from suitable solvent.

General procedure for the synthesis of isatin chalcones (11–14)

To a solid homogenous mixture of substituted isatin **(4–6)** (1 mmol) and acetophenones (**7–10**) (1 mmol) with catalytic

Fig. 2 Design strategy



amount of dimethylamine was taken in 250 mL conical flask and stirred for 15–30 min. The reaction mixture was cooled overnight, a colorless solid was formed. Twenty milliliter of glacial acetic acid and five drops of concentrated HCl was added to this precipitate and the mixture was warmed at 80 °C for 30 min and after dehydration, gave isatin chalcones (**11–14**).

General procedure for synthesis of various imines derivatives (15–19)

A mixture of 2-Amino-5-aryl-1, 3, 4-thiadiazole (**1–3**) (2 mmol) and substituted isatin chalcones (**11–14**) (2 mmol) was taken in 20 mL of absolute ethanol in 250 mL conical flask. The resulting mixture was refluxed for 5–8 h and the reaction mixture was cooled on overnight and solvent was evaporated under reduced conditions, the residue thus obtained was recrystallized from methanol gave imine derivatives **(15–19)**.

*3-(2-Phenyl-2-((5-phenyl-1,3,4-thiadiazol-2-yl)imino) ethylidene)indolin-2-one **(15)***

Yield 83%; m.p. 190–191 °C; IR (KBr) ṽ max cm^{-1}: 3426 (NH *str*), 3161 (C=CH, *str*), 1681 (C=O), 1650(C=N), 1458 (Ar–C=C), 1385(Ar–C=N), 1015 (Ar–C–S),.^{1}H NMR (300 MHz, DMSO-d_6) δ 9.96 (s, 1H), 8.93 (s, $J = 6.8$, 1H), 8.32–8.25 (m, 4H), 7.93–7.81 (m, 6H), 7.41 (t, $J =$ 7.2, 1H), 7.23 (t, $J = 7.2$, 1H), 7.06 (d, $J = 8.4$, 1H), 6.21(s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 191.0, 179.2 (2C), 169.3, 164.8, 143.2, 137.5, 136.6, 133.8 (2C), 132.7 (2C), 128.9 (4C), 128.0 (2C), 126.4 (2C), 122.9 (2C), 120.6, 110.2. ESI-MS *m/z*: 409.1 $[M+H]^+$; Anal. Calcd. for $C_{24}H_{16}N_4OS$: C, 70.57; H, 3.95; N, 13.72; found: C, 70.59; H, 3.98; N, 13.71.

*3-(2-((5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-yl)imino)-2-phenylethylidene)indolin-2-one **(16)***

Yield 80%; m.p. 189–190 °C; IR (KBr) ṽ max cm^{-1}: 3477 (NH *str*), 3106 (C=CH, *str*), 1681 (C=O), 1655 (C=N), 1385 (C=C), 1320 (Ar–C=N), 1015 (Ar–C–S), 735 (para-Cl). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.13 (s, 1H), 8.89 (s, $J = 7.2$, 1H), 8.34–8.26 (m, 4H), 7.89–7.83 (M, 5H), 7.43 (t, $J = 6.4$, 1H), 7.22 (t, $J = 7.2$, 1H), 7.09 (d, $J = 7.6$, 1H), 6.18 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.6, 178.8 (2C), 168.3, 163.9, 144.1, 139.3, 138.6, 136.3 (2C), 135.8, 133.3 (2C), 133.1 (2C), 132.4 (3C), 131.6, 126.5, 125.4, 122.8, 120.9, 110.8. ESI-MS *m/z*: 443.3 $[M+H]^+$; Anal. Calcd. for $C_{24}H_{15}ClN_4OS$: C, 65.08; H, 3.41; N, 12.65; found: C, 65.11; H, 3.48; N, 12.60.

*3-(2-Phenyl-2-((5-(p-tolyl)-1,3,4-thiadiazol-2-yl)imino) ethylidene) indolin-2-one **(17)***

Yield 78%; m.p. 186–187 °C; IR (KBr) ṽ max cm^{-1}: 3458 (NH *str*), 3106 (C=CH, *str*),2996 (C–H, *str*), 1681 (C=O), 1660 (C=N), 1446 (C=C), 1355 (Ar–C=N), 972 (Ar–C–S). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.09 (s, 1H), 8.84 (d, $J = 7.5$ Hz, 1H), 8.29–7.83 (m, 7H), 7.53–7.49 (m, 3H), 7.39 (t, $J = 7.6$ Hz, 1H), 7.13 (d, $J = 7.2$ Hz, 1H), 5.99 (s, 1H), 2.48 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6)

δ 188.3, 177.4, 168.9 (2C), 162.3, 138.8 (2C), 137.8, 137.1, 136.2, 133.6 (2C), 133.3 (2C), 131.6 (2C), 130.3, 129.5 (2C), 126.8, 125.3, 122.1, 120.3, 110.5, 21.6. ESI-MS *m/z*: 423.3 $[M+H]^+$; Anal. Calcd. for $C_{25}H_{18}N_4OS$:C, 71.07; H, 4.29; N, 13.26; found: C, 71.15; H, 4.32; N, 13.29.

3-(2-(4-Hydroxyphenyl)-2-(5-phenyl-1,3,4-thiadiazol-2-ylimino)ethylidene)indolin-2-one ***(18)***

Yield 80%; m.p. 182–183 °C; IR (KBr) $\tilde{\nu}$ max cm^{-1}: 3456 (NH *str*), 3300 (OH, *str*), 3100 (C=CH, *str*), 1670 (C=N), 1385 (C=C), 1320 (Ar–C=N), 1015 (Ar–C–S), ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.98 (s, 1H), 8.89–8.86 (m, 3H), 8.39 (d, $J = 6.8$ Hz, 2H), 8.10–7.89 (m, 4H), 7.43 (t, $J = 7.6$ Hz, 1H), 7.16 (d, $J = 7.6$ Hz, 1H), 6.91 (d, $J = 6.8$ Hz, 2H), 6.01 (s, 1H), 5.39 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 191.5, 178.4, 169.1, 165.3, 163.9, 144.2, 138.9, 136.8 (2C), 136.2 (3C), 134.1 (2C), 132.5, 131.3, 127.2, 126.2, 125.1, 124.2, 120.6, 116.7 (2C), 110.8. ESI-MS *m/z*: 425[M +H]. Anal. Calcd. for $C_{24}H_{16}N_4O_2S$.C, 67.91; H, 3.80; N, 13.20; found: C, 67.98; H, 3.86; N, 13.18.

3-(2-((5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-yl)imino)-2-(4-hydroxyphenyl) ethylidene)indolin-2-one ***(19)***

Yield 80%; m.p. 218–219 °C; IR (KBr) $\tilde{\nu}$ max cm^{-1}: 3495 (NH *str*), 3350 (OH, *str*), 3110 (C=CH, *str*), 1685 (C=N), 1385 (C=C), 1365 (Ar–C=N), 926 (Ar–C–S), 735 (para-Cl). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.02 (s, 1H), 8.78–8.74 (m, 3H), 8.34(d, $J = 7.2$ Hz, 2H), 8.13–7.93 (m, 3H), 7.36 (t, $J = 7.6$ Hz, 1H), 7.09 (d, $J = 7.2$ Hz, 1H), 6.88 (d, $J = 8.4$ Hz, 2H), 6.03 (s, 1H), 5.36 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 189.3, 179.3 (2C), 170.8, 165.5, 154.3, 149.8, 138.9 (2C), 136.3, 134.6 (2C), 133.8 (2C), 132.6 (2C), 131.3, 128.4, 127.1, 126.2, 123.6, 121.4, 116.9, 111.2. ESI-MS *m/z*: 459.7 $[M+H]^+$; Anal. Calcd. for $C_{24}H_{15}ClN_4O_2S$: C, 62.81; H, 3.29; N, 12.21; found: C, 62.87; H, 3.31; N, 12.15.

General procedure for the synthesis of 5-substituted-5′-substituted phenyl-2′,4′-dihydrospiro[indoline-3,3′-pyrazol]-2-one derivatives (20–24)

A mixture of substituted isatin chalcones **11–14** (1 mmol) and hydrazine hydrate (1 mmol) was taken in 30 mL of absolute ethanol in 25 mL conical flask. Then the reaction mixture was refluxed for about 1 h. Then the reaction mixture was cooled on overnight and the precipitate was collected by filtration. Thus, obtained spiro derivatives **(20–24)** were purified by column chromatography and subsequently by recrystallization with absolute ethanol.

5′-Phenyl-2′,4′-dihydrospiro[indol-3,3′-pyrazol]-2-one ***(20)***

Yield 84%; m.p. 200–201 °C; IR (KBr) $\tilde{\nu}$ max cm^{-1}: 3481 (NH *str*), 3422 (NH *str*), 1709 (C=O), 1600 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.16 (s, 1H), 8.09 (s, $J = 7.2$ Hz, 1H), 7.63 (d, $J = 7.2$ Hz, 2H), 7.56–7.51 (m, 3H), 7.22–7.16 (m, 2H), 6.92 (t, $J = 7.6$ Hz, 1H), 6.20 (s, 1H), 3.43 (d, $J = 15.0$ Hz, 1H), 3.72 (d, $J = 15.0$ Hz, 1H). ^{13}C NMR (75 MHz, DMSO- d_6) δ 180.3, 151.3, 140.1, 132.5, 130.9, 130.1, 129.3, 129.0, 128.3, 128.4, 127.6, 126.3, 122.4, 113.8, 70.3, 45.9. ESI-MS *m/z*: 264.1 $[M+H]^+$; Anal. Calcd. for $C_{16}H_{13}N_3O$: C, 72.99; H, 4.98; N, 15.96; found: C, 72.87; H, 5.02; N, 15.93.

5′-(4-Chlorophenyl)-2′,4′-dihydrospiro[indoline-3,3′-pyrazol]-2-one ***(21)***

Yield 71%; m.p. 222–223 °C; IR (KBr) $\tilde{\nu}$ max cm^{-1}: 3461 (NH, *str*), 3279 (NH *str*), 1712 (C=O), 1609 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ9.32 (s, 1H), 8.02 (s, $J = 7.6$ Hz, 1H), 7.64 (t, $J = 6.8$ Hz, 2H), 7.46 (d, $J = 8.4$ Hz, 2H), 7.26–7.21 (m, 2H), 6.97 (t, $J = 7.6$, 1H), 6.33 (s, 1H), 3.58 (d, $J = 15.0$ Hz, 1H), 3.43 (d, $J = 15.0$ Hz, 1H). ^{13}C NMR (75 MHz, DMSO- d_6) δ 178.5, 147.5, 141.7, 133.9, 132.3 (2C), 129.7 (2C), 128.9, 127.8 (2C), 124.0, 122.8, 110.1, 70.1, 44.1.ESI-MS *m/z*: 298.5 $[M+H]^+$; Anal. Calcd. for $C_{16}H_{12}ClN_3O$: C, 64.54; H, 4.06; N, 14.11; found: C, 64.47; H, 4.02; N, 14.02.

1-Benzyl-5′-phenyl-2′,4′-dihydrospiro[indoline-3,3′-pyrazol]-2-one ***(22)***

Yield 71%; m.p. 232–235 °C; IR (KBr)$\tilde{\nu}$ max cm^{-1}: 3476 (NH*str*), 3276 (NH *str*), 1706 (C=O), 1603 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ9.19 (s, 1H),7.71 (d, $J = 7.2$ Hz, 2H), 7.61–7.55 (m, 3H), 7.41–7.25 (m, 8H), 6.98 (d, $J = 6.8$, 1H), 4.66 (s, 2H), 3.58 (d, $J = 15.0$ Hz, 1H), 3.46 (d, $J = 15.0$ Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 177.3, 147.8, 142.9, 141.8, 131.8, 131.6, 130.2, 129.9, 125.6 (2C), 125.3 (2C), 125.0 (2C), 124.7, 123.7(3C), 123.2, 116.4, 67.1, 54.6, 44.8.ESI-MS *m/z*: 354.3 $[M+H]^+$; Anal. Calcd. for $C_{23}H_{19}N_3O$: C, 78.16; H, 5.42; N, 11.89; found: C, 78.12; H, 5.48; N, 11.87.

5-Bromo-5′-phenyl-2′,4′-dihydrospiro[indoline-3,3′-pyrazol]-2-one ***(23)***

Yield 83%; m.p. 232–234 °C; FTIR (KBr) $\tilde{\nu}$ max cm^{-1}: 3462 (NH, *str*), 3278 (NH, *str*), 1707 (C=O), 1618 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6), 9.12 (s, 1H), 8.11 (s, $J = 7.5$ Hz, 1H), 7.61 (d, $J = 8.8$ Hz, 2H), 7.53–7.47 (m, 3H), 7.21 (d, $J = 7.6$ Hz, 1H), 7.02 (d, $J = 7.2$ Hz, 1H), 6.89 (s, 1H), 3.43 (d, $J = 15.0$ Hz, 1H), 3.34 (d, $J = 15.0$ Hz, 1H).

^{13}C NMR (75 MHz, DMSO-d_6) δ 178.2, 147.6, 147.2, 131.8, 130.1, 128.9, 128.6, 127.1, 123.8 (2C), 123.1 (2C), 122.6, 120.8, 68.4, 43.4.ESI-MS *m/z*: 343.1 $[M+H]^+$; Anal. Calcd. for $C_{16}H_{12}BrN_3O$.C, 56.16; H, 3.53; N, 12.28; found: C, 56.20; H, 3.58; N, 12.20.

5′-(4-Methoxyphenyl)-2′,4′-dihydrospiro[indoline-3,3′-pyrazol]-2-one ***(24)***

Yield 83%; m.p. 232–234 °C; IR (KBr) ṽ max cm^{-1}: 3438 (NH, *str*), 3391 (NH *str*), 1710 (C=O), 1617 (C=N) , 1301 (OCH_3). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.02 (s, 1H), 7.64 (d, $J = 8.8$ Hz, 2H), 7.33 (d, $J = 7.6$ Hz, 1H), 7.28 (d, $J = 6.8$ Hz, 1H), 7.05 (t, $J = 7.6$ Hz, 1H), 6.91 (t, $J = 10.0$ Hz, 3H), 6.02 (s, 1H), 3.84 (s, 3H), 3.72 (d, $J = 15.0$ Hz, 1H), 3.42 (d, $J = 15.0$ Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 178.7, 159.5, 147.4, 141.4, 132.3, 129.0, 127.1 (2C), 125.3, 123.5, 122.1, 114.0 (2C), 109.6, 69.5, 56.1, 43.9.ESI-MS *m/z*: 294.2 $[M+H]^+$; Anal. Calcd. for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33; found: C, 69.68; H, 5.12; N, 14.39.

Molecular docking studies

To gain more insight about the binding modes of synthesized derivatives (**15–24**), we herein performed docking studies (Rekulapally et al. 2015) on Epidermal Growth Factor Receptor (EGFR). In this study, X-ray crystal structure of human EGFR at resolution 1.9 Å (PDB ID: 4ICZ) was used further to identify binding modes involved in the inhibition activity. The protein monomer was optimized for geometry correction followed by energy optimization using Merck Molecular Force Field (MMFF). This optimized receptor was used for docking simulation. Molecular docking studies for synthesized analogues were carried out using GRIP batch docking method available in BioPredicta tools of VLife Molecular Design Suite 4.3 software. GRIP docking employees the PLP scoring function for ligand receptor interactions (i.e., hydrogen bonding, steric interactions, vanderwaal's interactions, hydrophobic interactions and electrostatic interactions) with the active site of EGFR protein. The PLP scores were compared with gefitinib (Pao et al. 2004), an EGFR inhibitor used for breast, lung, and other cancers. The best conformers and the dock score for each ligand was shown in Table 1.

Antitumor activity

The cytotoxic activity of synthesized compounds was evaluated at National Cancer Institute (NCI), Bethesda, Maryland, USA in an in vitro 60 human tumor cell lines panel. The human tumor cell line derived from nine

Table 1 Dock scores [PLP] of 3-(2-(p-substituted)-2-((5-phenyl-1,3,4-thiadiazol-2-yl)imino)-2-(p-substituted)ethylidene)indolin-2-one (15–19) and 5-substituted-5′-substituted phenyl-2′,4′-dihydrospiro[indoline-3,3′-pyrazol]-2-one derivatives (20–24) and gefitinib

Ligands	Dock score	Vanderwaals	Interacting residues		
			Hydrogen	Hydrophobic	Charge
15	−79.11	Gly 326, Asn 327, Asn 381, Lys 330, Thr 355, Thr 382, Tyr 357, Phe 426	Asn 327	–	–
16	−64.18	Asn 327, Lys 330, Tyr 357, Glu 380, Asn 381, Thr 382, Arg 384, Arg 425, Asp 385, Phe 426, Gly 427	–	–	–
17	−61.25	Gly 326, Gly 427, Glu 329, Lys 330, Gln 354, Thr 355, Thr 382, Tyr 357, Asn 381, Arg 384, Asp 385, Phe 426,	–	Arg 384, Phe 426, Gly 427	–
18	−62.34	Arg 349, Gln 354, Glu 380, Asn 381, Arg 384, Asp 385, Phe 426	Asp 385	–	–
19	−69.84	Gly 326, Glu 329, Leu 346, Thr 355, Tyr 357, Asn 381, Thr 382, Arg 384, Asp 385, Phe 426	Thr 355	–	–
20	−52.49	Gly 326, Asn 327, Glu 329, Lys 330, Gln 354, Thr 355, Tyr 357, Asn 381, Asp 385	Thr-1	Asn 381	Gln 354
21	−59.45	Gly 326, Asn 327, Glu 329, Lus 330, Thr-1, Thr 355, Gln 354, Tyr 357, Asn 381, Asp 385,	Thr 355	Gln 354	Gln 354, Thr 355
22	−62.08	Gly 326, Glu 329, Arg 349, Thr-1, Asn 381, Thr 382, Arg 384, Asp 385, Phe 426,	Asn 381	Thr-1, Asn 381	Asn 381, Thr 382
23	−47.17	Gly 326, Asn 327, Lys 330, Thr-1, Gln 354, Tyr 357, Glu 380, Asn 381, Asp 385	Thr-1	–	Thr-1, Gln 354, Thr 355
24	−54.09	Thr-1, Gln 354, Thr 355, Glu 380, Asn 381, Thr 382, Tyr 383, Arg 384, Asp 385, Phe 426,	Thr 355	Tyr 383, Arg 384	Lys 330, Asn 381
Gefitinib	−71.05	Glu 380, Asn 381, Gly 326, Thr 355, Tyr 357, Thr 382, Asp 385, Arg 425, Phe 426, Gly 427,	Asn 381,	Tyr 383, Arg 384	Gly 427, Phe 426

neoplastic cancer types i.e., leukemia, non-small cell lung, prostate, melanoma, breast, colon, CNS, ovarian, and renal cancers. In vitro cytotoxic assays were performed according to the USA NCI protocol. The compounds were first evaluated at single dose primary anticancer assay towards 60 cancer lines (concentration 10^{-5} M). Compounds which exhibit significant growth inhibition are evaluated against the 60 cell panel at five concentration levels (Andreani et al. 2008; Grever et al. 1992; Shoemaker 2006; Alley et al. 1988).

Results and discussion

Chemistry

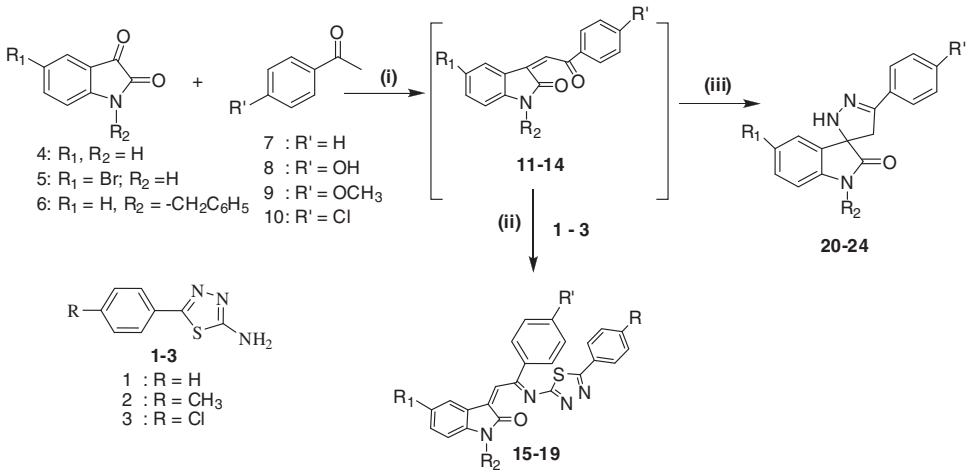
As a starting point for the study, new derivatives were synthesized from the intermediate isatin chalcones (**11–14**), which were prepared by the reaction of acetophenones (**7–10**) with isatin (**4–6**) in a solvent free condition using dimethylamine by refluxing in glacial acetic acid and Conc HCl. The compounds isatin-thiadiazole hybrids (**15–19**) were synthesized from isatin chalcones (**11–14**) and 2-amino-5-aryl-thiadiazoles (**1–3**). The 2-amino-5-aryl-thiadiazole was synthesized from benzyladehyde and thiosemicarbazide under reflux to give thiosemicarbazone and subsequently cyclized with bromine in acetic acid. The synthesis of spiro isatin-pyrazolines (**20–24**) was carried as reported earlier in the literature (Mohammadizadeh 2006) (Scheme 1)

Derivatives obtained were fully characterized by FT-IR, ^{1}H and ^{13}C NMR and Mass (ESI) spectral data. All compounds have shown an excellent agreement between calculated and experimentally obtained data for CHN analysis.

The IR spectrum of compounds isatin-thiadiazole hybrids (**15–19**) exhibited absorption bands in the range of 3200–3100 cm^{-1} due to -ene C–H stretching bonds, 1650–1500 cm^{-1} due to imine C=N stretching and 800–700 cm^{-1} due to aromatic deformation. The ^{1}H NMR peaks in the range of δ 8.93–6.88 ppm were due to different aromatic protons. δ5.0–6.0 ppm was characteristic to =CH protons; and around δ 9.0–11.0 ppm was assigned to –NH proton of isatin. The ^{1}H NMR spectrum of compounds **20–24** exhibited two doublets (δ 3.42–3.84 ppm) due to $-CH_2$ protons of pyrazoline ring and multiplets (δ 6.5–8.00 ppm) for the aromatic protons. The singlet protons at around δ 9.16 and 6.20 are assigned for –NH proton of isatin and pyrazoline, respectively.

Molecular docking

The synthesized compounds **15–24** were docked with 4ICZ binding site of EGFR protein wherein some of the compounds showed better docking score than the standard drug gefitinib. Docking results showed that compound 3-(2-phenyl-2-((5-phenyl-1,3,4-thiadiazol-2-yl) imino)ethylidene) indolin-2-one (**15**) has highest dock score of −79.11 with hydrogen bond and vanderwaals interactions between protein and ligand. VLife Sciences 4.3 was employed for the docking studies to explore the binding mode of ligands. To validate the docking simulations, gefitinib was used as the reference ligand. The original ligand score obtained for gefitinib was −71.05, confirming the ability of the method to accurately predict the binding confirmation. All ligands exhibited negative docking scores and were comparable with the reference gefitinib. From the dock score, compounds **15, 16, 17, 18, 19**, **21**, and **22** were found to have highest negative dock score ranging from



Scheme 1 Synthesis of isatin based pyrazolines and thiadiazolines. *Reaction conditions*: (i) dimethylamine, glacial acetic acid, Conc HCl, reflux, 80 °C, 30 min; (ii) substituted 2-amino5-aryl thiadiazole (**1–3**), EtOH, reflux, 5–8 h, (iii) hydrazine hydrate, EtOH, reflux, 1 h

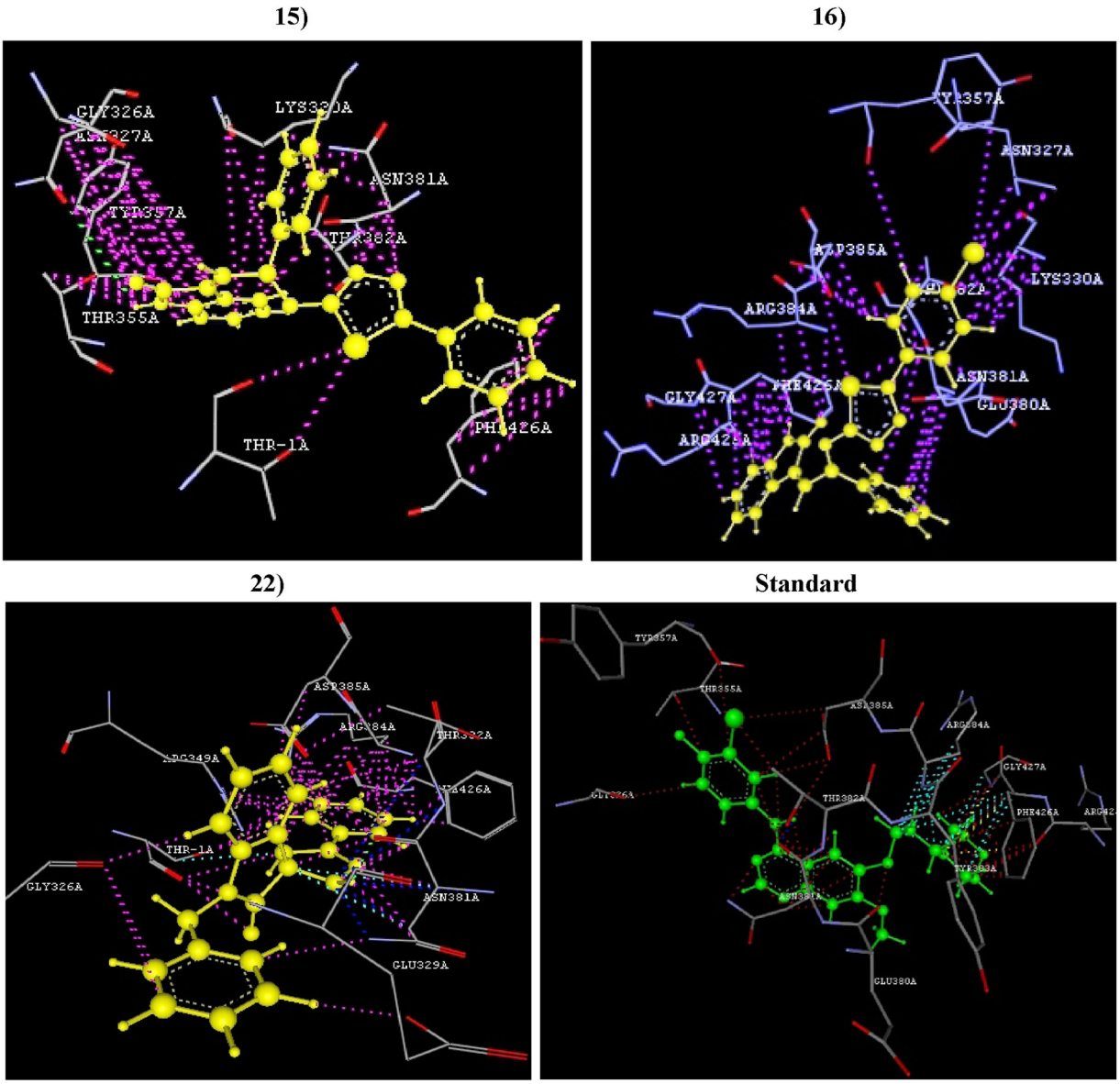


Fig. 3 3D interactions of ligands 15, 16, 22 and gefitinib showing hydrogen, charge, hydrophobic and vanderwaal's interactions with EGFR protein

−79.11 to −59.45 (Table 1). All the docked compounds were analyzed for various types of interactions such as hydrophobic bonding, charge, hydrogen, and vanderwaal's interactions. Figure 3 shows the docking of the ligands in the receptor cavity. All the ligands and reference exhibited same interactions such as hydrogen, charge, hydrophobic, and vanderwaal's interactions as shown in Table 1.

Antitumor activity

Seven newly synthesized compounds (**15, 16, 20, 21, 22, 23,** and **24)** were selected by National Cancer Institute (NCI) Developmental Therapeutic Program (http://www.dtp.nci.nih.gov), Bethesda, MD, U.S.A. All the derivatives were subjected to the NCI's disease oriented human cell lines screening assay for the evaluation of their in vitro antitumor activity. The compounds were tested at a single-dose concentration of 10 μM, and the percentage of growth over the 60 tested cell lines were determined and illustrated in (Table 2).

Among all, 3-(2-(p-substituted)-2-((5-phenyl-1,3,4-thiadiazol-2-yl)imino)-2-(p-substituted)ethylidene)indolin-2-one derivatives (**15** and **16**) and 5-substituted-5′-substituted phenyl-2′,4′-dihydrospiro[indoline-3,3′-pyrazol]-2-one (**20–24**) analogs showed a distinctive pattern of selectivity. Compound 3-(2-phenyl-2-((5-phenyl-1,3,4-thiadiazol-2-yl)

Table 2 Sixty human tumor cell lines anticancer screening data of synthetic analogues

Growth percent in one-dose assay (The most sensitive cell lines)							
Panel/cell line	**15** NSC 767490	**16** NSC 767496	**20** NSC 767491	**21** NSC 767492	**22** NSC 767493	**23** NSC 767494	**24** NSC 767495
Leukemia							
CCRF-CEM	−45.05	−42.66	49.17	88.3	84.37	32.34	102.86
HL-60(TB)	−32.88	−31.81	−8.55	99.13	87.78	−4.96	100.42
K-562	20.39	8.67	18.49	94.54	81.14	23.76	96.68
MOLT-4	−24.19	−26.44	38.71	83.78	81.82	36.34	94.72
RPMI-8226	−17.47	−14.37	47.28	77.78	83.45	28.3	82.75
SR	−22.97	−27.6	−7.86	86.22	73.01	12.5	91.5
Non-small cell lung cancer							
NCI-H522	−59.34	−95.65	24.58	90.07	88.89	20.02	95.86
Colon Cancer							
COLO 205	2.53	0.63	31.37	108.37	106.75	30.54	100.05
HCT-116	−51.94	−58.54	29.39	95.32	102.06	22.9	87.96
HT29	−36.27	−56.29	8.14	97.12	106.72	12.15	106.59
Melanoma							
LOX IMVI	−86.29	−79.3	48.36	99.48	97.18	51.91	101.88
Renal cancer							
786–0	−19.27	−56.92	61.72	100.24	97.1	46.83	102.31
ACHN	−54.79	−71.4	59.35	91.04	99.71	49.06	90.88
UO-31	−87.25	−69.15	57.58	69.22	103.54	48.4	82.51
Mean	34.61	20.82	48.18	96.33	99.48	42.38	98.39
Delta	121.86	116.47	73.11	27.11	31.02	63.47	16.57
Range	193.33	199.41	115.56	47.14	57.41	103.08	43.81

imino)ethylidene) indolin-2-one (**15**) and 3-(2-((5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)imino)-2-phenyl ethylidene) indolin-2-one (**16**) showed remarkably lowest cell growth promotion against Non-small lung NCI-H522 cancer cell line with cell growth promotion of −59.34 and −95.65 respectively and also showed lethality against Melanoma LOX IMVI cancer cell line with −86.29 and −79.30 respectively. Lowest cell growth promotion are also observed by compound **15** against Leukemia CCRF-CEM (−45.05), HL-60 (−32.88), MOLT-4 (−24.19), RPMI-8226 (−17.47), and SR (−22.97); Colon cancer HCT-116 (−51.94). The compound **16** also showed significant cytotoxic activity against Leukemia CCRF-CEM (−42.66), HL-60 (−31.81), MOLT-4 (−26.44), RPMI-8226 (−14.37), and SR (−27.60) Colon cancer HCT-116 (−58.54). Compounds **15, 16, 20** and **23** showed growth promotion values of 2.53, 0.63, 31.37, and 30.54, against Colon COLO 205 respectively; while compound **20** and **23** showed values of 8.14 and 12.15 against Colon HT29. Through evaluation of the data revealed in (Table 2) showed compounds **15, 16, 20,** and **23** are the most potent of this study, displaying usefulness towards various cell types belong to distinct tumor subpanels. Compounds **15, 16, 20,** and **23** showed

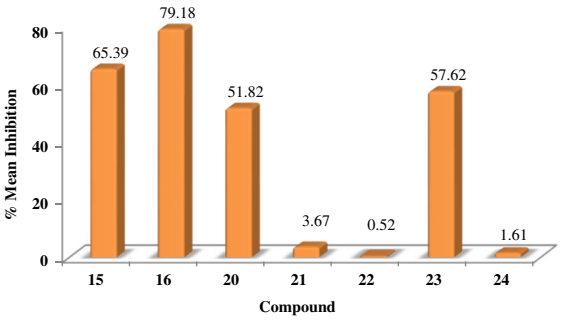


Fig. 4 Mean inhibition percentages of the compounds in single dose (10 μM)

higher percent mean inhibition than **21, 22,** and **24.** Compound with chloro substitution on phenyl ring was more active than compound **15** with unsubstituted phenyl ring. Also compound **20** and **23** were more active than **21, 22,** and **24** (Fig. 4).

Compounds **15**, **16**, **20,** and **23** passed the prime anticancer assay at strength of 10 μM. Subsequently, these active compounds tested towards a panel of sixty distinct tumor cell types at a 5-log dose range. Two of these four analogues were selected for a repeat screen (**15** and **16**) and the

Table 3 GI_{50} values (μM) of the tested compounds over the most sensitive cell line of each subpanel

Compound No	Cancer cell lines										
	CCRF-CEM[a]	HOP-92[b]	NCI-522[b]	HCT-116[c]	SF-295[d]	SNB-75[d]	MDA-MB-435[e]	OVCAR-3[f]	A498[g]	PC-3[h]	BT-549[i]
15*	1.51	0.99	0.65	1.35	17.09	3.72	2.96	1.39	9.85	2	1.92
16*	1.17	0.97	0.72	1.07	12.17	2.53	2.57	1.28	10.51	1.93	1.8
20	5.49	1.65	4.66	4.83	4.84	2.98	1.66	5.1	1.85	3.89	6.35
23	3.63	1.66	3.56	4.27	2.77	1.35	4.64	3.26	2.24	2.28	1.3

* Values are average of two runs; [a] Leukemia cell lines; [b] Non-small Cell lung cancer cell lines; [c] Colon cancer cell lines; [d] CNS cancer cell lines; [e] Melanoma cell lines; [f] Ovarian cancer cell lines; [g] Renal cancer cell lines; [h] Prostate; [i] Breast cancer cell lines

results obtained are shown in Table 3, as the average of these two runs. Compounds **15, 16, 20,** and **23** showed remarkable broad-spectrum antitumor activity.

The entire cell lines (about 60), representing nine tumor subpanels were incubated at five different concentrations (0.01, 0.1, 1, 10, and 100 μM). Three response parameters GI_{50}, TGI, and LC_{50} were calculated for each cell line. Tested compounds **15, 16, 20,** and **23** displayed effective growth inhibition GI_{50} (MG-MID) values of 3.01, 2.81, 6.02, and 4.07 μM, respectively, beside cytostatic activity TGI (MG-MID) values of 8.70, 8.51, 69.18, and 30.9 μM, respectively (Fig. 5). In addition, they exhibited some cytotoxic activity with LC_{50} (MG-MID) values of 29.5, 30.9, 100, and 95.4 μM, respectively as shown in Fig. 5.

Compound 3-(2-phenyl-2-((5-phenyl-1,3,4-thiadiazol-2-yl) imino)ethylidene) indolin-2-one **(15)** demonstrated remarkable anticancer activity towards almost all of the tested cell lines on behalf of nine distinct subpanels with GI_{50} values between "0.65–9.85 μM", expect two cell lines SF-295 and SNB-19 of CNS cancer subpanel showing GI_{50} at a concentration of 17.09 and 12.29 μM respectively. The results are shown in Table 3, with regard to sensitivity against some individual cell lines the compound showed high activity against Non-Small Cell Lung Cancer NCI-H522 and HOP-92 with GI_{50} 0.65 and 0.99 μM respectively. GI_{50}, TGI, and LC_{50} MG-MID values of 3.01, 8.70, and 29.5 μM respectively, proved to be the most active member in this study. On the other hand, 3-(2-((5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)imino)-2-phenyl ethylidene) indolin-2-one **(16)** showed nearly the same pattern of activity as **15** but to a lesser extent. This compound is active towards non-small cell lung cancer (NCI-H522 and HOP-92) and colon (HCT-116) with GI_{50} values 0.72, 0.97, and 1.07 μM respectively. The results are presented in Table 3.

5-Bromo-5′-phenyl-2′,4′-dihydrospiro[indoline-3,3′-pyrazol]-2-one **(23)** is active against CNS cancer (SNB-75), colon cancer (HCC-2998), and non-small lung cell cancer (HOP-92) showing GI_{50} values 1.35, 1.59, and 1.66 μM respectively. The results of compound **23** at five dose level in μM are shown in Table 3. The compounds displayed relatively weaker growth inhibition, cytostatic, and cytotoxic patterns when compared with compound **15** and compound **16** (GI_{50}, TGI, and LC_{50}MG-MID values 4.07, 30.9, and 95.49 μM, respectively). Finally, 5′-phenyl-2′,4′-dihydrospiro[indoline-3,3′-pyrazol]-2-one **(20)** active against Melanoma (MDA-MB-435), non-small cell lung cancer (HOP-92) and renal cancer (A498) with GI_{50} values 1.66, 1.65, and 1.85 μM respectively. The compound **20** shows significant activity against all cancer cell lines except on ovarian (OVCAR-4 and OVCAR-5) and non-small cell lung cancer (NCI-H226). The results are shown in Table 3. The compound has shown weaker growth inhibition, cytostatic, and cytotoxic patterns with GI_{50}, TGI, and LC_{50} MG-MID values 6.02, 69.18, and 100 μM (Fig. 5).

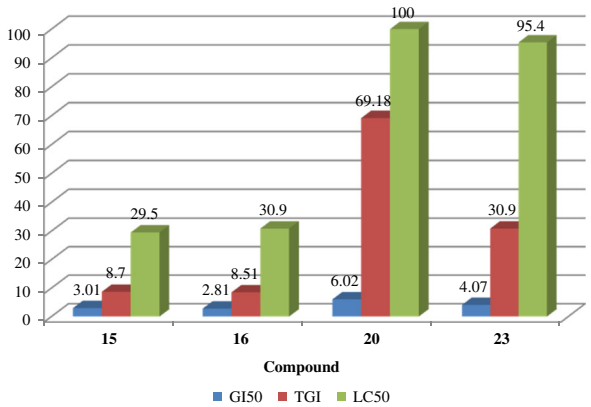


Fig. 5 GI_{50}, TGI, LC_{50}MG-MID of in vitro cytotoxicity data for compounds 15, 16, 20, and 23 against human tumor cell lines

Conclusion

In conclusion, we have synthesized a new series of isatin based thiadiazoline and pyrazoline derivatives as a novel class of antitumor agents. Among these compounds 3-(2-Phenyl-2-((5-phenyl-1,3,4-thiadiazol-2-yl)imino)ethylidene) indolin-2-one **(15)** and 3-(2-((5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)imino)-2-phenylethylidene)indolin-2-one **(16)** displayed significant selective cytotoxic activity against non-

small cell lung cancer (NCI-H522) with GI_{50} 0.65 and 0.72 μM respectively. Thus, structure-activity investigation revealed that isatin-thiadiazole conjugates displayed high significant activity compared to that of spiroisatin–pyrazoline derivatives. Docking studies performed on the synthesized compounds by using VLife Molecular Design Suite 4.3 software, has confirmed that inhibitors fit into the binding pocket of the EGFR, 4ICZ protein. From the results, we found that for successful docking, hydrogen bonding and hydrophobic interactions between the ligand and the receptor are very important. Further studies will be undertaken to elucidate the antitumor mechanism of action involved.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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