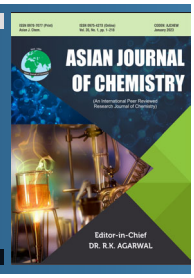
Asian Journal of Chemistry; Vol. 35, No. 3 (2023), 705-711

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2023.27297




# Microwave-Assisted Synthesis, Molecular Docking Studies and Biological Evaluation of Novel Thiazole, Imidazole-Indole Hybrids

MOHD AFROZ*, and G. SHIVA KUMAR

GITAM School of Pharmacy, GITAM (Deemed to be University), Sangareddy-502329, India

*Corresponding author: E-mail: mohdafroz1992@gmail.com

*Received*: 24 December 2022; *Accepted*: 6 February 2023; *Published online*: 27 February 2023; AJC-21164

The present work deals with the synthesis of novel thiazole, imidazole-indole hybrids **3a-l** using microwave assisted method as a green chemistry approach. The title compounds were synthesized in a good yield between 68-85%. All the synthesized structures were confirmed by FT-IR, LC-Mass, $^{1}$H NMR and $^{13}$C NMR analytical data. All the novel indole derivatives were screened for anticancer activity by MTT assay method against the MCF-7 and SKVO3 cell lines. Compound **3i** ($IC_{50}$ value of 26.98 µg, 36.12 µg) and **3j** ($IC_{50}$ value of 24.11 µg, 38.43 µg) exhibited good anticancer activity as compared to doxorubicin. Further, the synthesized compounds were also screened for antibacterial activity using agar diffusion method, where compounds **3c**, **3d**, **3f** and **3j** exhibited good antibacterial activity. Furthermore, the binding pattern and affinity of these new indole towards the active region of EGFR were studied using molecular docking studies and the results were in good agreement with the biological results.

**Keywords: Indole, Imidazole, Molecular docking, Antibacterial activity, Anticancer activity, MCF-7 cell, SKVO3 cell.**

## INTRODUCTION

In heterocyclic chemistry, microwave method plays a major role in the progress of developing chemistry and it became a challenging to medicinal chemist [1]. Microwave methods and their utility and its scaffolds shows a diverse pharmacological and therapeutic activity. In synthetic chemistry, five, six and seven-membered nucleus has diversified and potential to various biologically activity [2,3]. Biological systems are inhibited by the molecular binding of heterocyclic compounds like indole, imidazole and thiazole because of their lone-pair effect and potent electron-withdrawing group. It has potentiality to bind the receptor and shows as agonist effect to receptor. It has wide-linked properties like tubulin polymerization inhibitor and apoptosis inducer with other hetero nucleus scaffolds known in the literature for their potent compound with a wide range of clinically and therapeutic properties [4-6].

Due to their privileged and efficacy towards pharmacological properties. Fused heterocyclic moieties like indole, thiazole, imidazole and indole ring structure are important in the drug discovery process. All these core structural moiety having numerous pharmacological and clinically properties such as anticancer, anti-inflammatory, antihypertensive α-blocking, anti-leishmanial, neuroprotective agents in Alzheimer and Parkinson-disease [7-11].

Since these thiazole, imidazole-indole hybrids are so beneficial as biologically active molecules in medicinal chemistry, we designed this study to synthesize new, novel thiazole, imidazole-indole hybrids scaffolds using Schiff and Mannich base mechanism and found them to be promising against the human ovarian cancer cell lines MCF-7 and SKOV3. Moreover, *in silico* molecular docking studies and interaction with protein ligand dataset and every docked dataset was also generated *via* Glide XP module [12].

## EXPERIMENTAL

Chemicals and reagents were purchased from SD Fine and Aura Laboratories. The synthesis compounds were characterized by TLC methods (silica gel plates) The TLC was carried out by using mobile phase *n*-hexane an ethyl acetate (6:4). The synthesized compounds were characterized using spectral techniques like FTIR (Shimadzu), $^{1}$H NMR (300 MHZ) solvent DMSO-$d_6$, $^{13}$C NMR (300 MHZ) solvent DMSO-$d_6$ and mass spectrometry (Shimadzu). Finally, the molecular docking studies was carried out by using Schrödinger software.

## Synthesis

**Cyclization reaction (step-1): Synthesis of substituted 2-amino-4-aryl thiazole (1a-d):** A mixture of equimolar quantity of substituted acetophenone (0.1 mol) and iodine (0.1 mol) was taken in an open vessel contain Teflon coated stir bar followed by the addition of 0.2 mol of thiourea. The vessel was placed microwave cavity (CEM, Discover) and subjected to microwave irradiation at 140 ºC for 10 min with 12 s time interval. After the completion of the reaction, cool the crude mixture at 70 ºC and then it was triturated to get precipitate. The crude product was filtered, washed with excess amount of ethanol followed by the addition of hot water. The pH was adjusted to 11-12 with $NH_4OH$ solution to get the precipitate. Finally, the precipitate was filtered and recrystallized by using a mixture of ethanol and water (1:4).

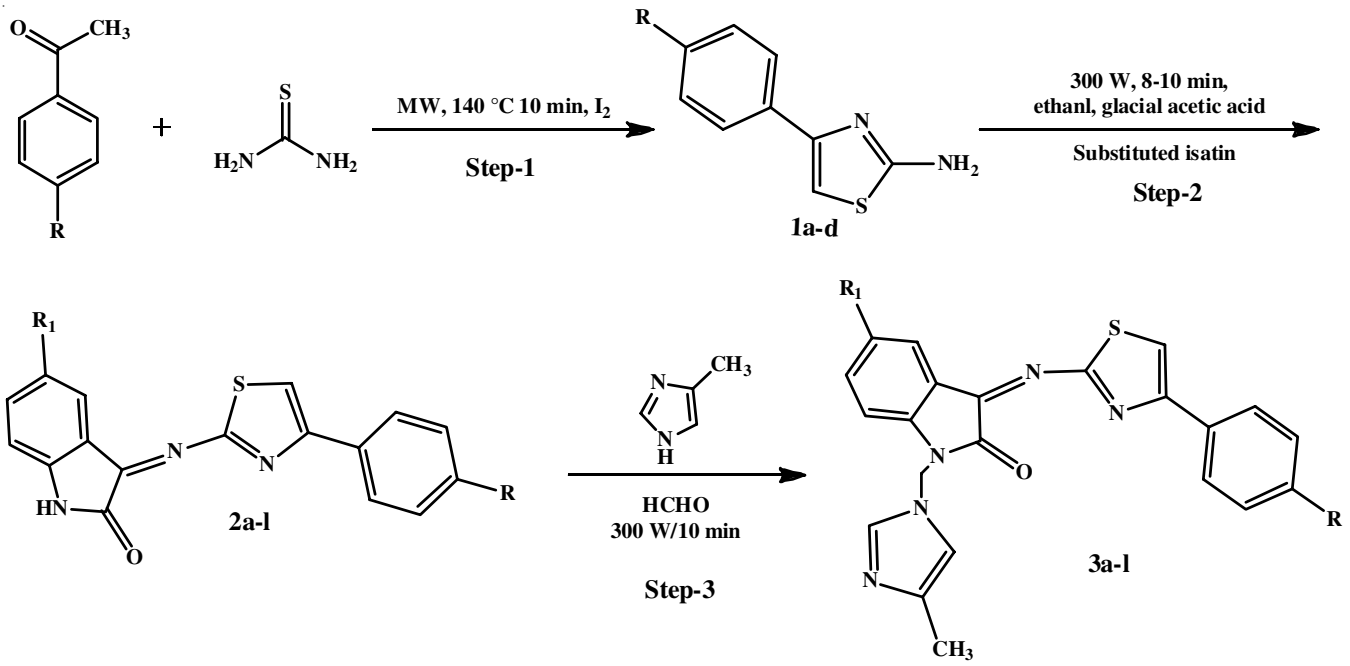
**Step-2: Synthesis of substituted isatin Schiff bases (2a-l):** Equimolar quantity of substituted 2-amino-4-aryl thiazole (**1a-d**, 0.1 mol) and substituted isatin (0.01 mol) were mixed in an open vessel followed by the addition of 20 mL of absolute ethanol and 10 mL of glacial acetic acid. The reaction was carried out under the microwave irradiation at 300 W for 8-10 min. The completion of the reaction was checked by TLC [*n*-hexane and ethyl acetate (7:3)] and the reaction mixture was cooled to room temperature and the solvent was removed by distillation to form final product. The crude product was recrystallized by using ethanol.

**Step-3: Synthesis of substituted 1-((4-methl-1*H*-imidazol-1-yl)methyl)-3-((4-phenyl thiazol-2-yl)imino)indolin-2-one (3a-l)**: Substituted isatin Schiff bases (**2a-l**, 0.1 mol) in 10 mL of absolute ethanol, formaldehyde (0.1 mol, 1 mL) and 4-methyl imidazole (0.11 mol) were mixed in an open vessel containing a Teflon coated stir bar. The reaction mixture was carried out under the microwave assisted method at 300 W for 10 min. Then the completion of the reaction mixture was kept on refrigerator for overnight. The obtained product was filtered and recrystallized by ethanol (**Scheme-I**). Finally, the pure title compounds were obtained by column chromatography [silica gel and ethyl acetate:hexane (3:1)].

**1-((4-Methyl-1*H*-imidazol-1-yl)methyl)-3-((4-phenylthiazol-2-yl)imino)indolin-2-one (3a):** Yield: 78%, m.p.: 159-161 ºC. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3070 (C-H *str.*, Ar-H), 2951, 2812 (C-H *str.*, aliphatic –CH-), 2282 (-CSC- *str.*), 1712 (C=O *str.*, isatin), 1623 (-C=N *str.*), 1563 (-C=CH *str.*), 1099 (C-N *str.*). $^1$H NMR (DMSO) δ ppm: 7.8918-7.8310 (d, 2H, Ar-H), 7.7958-7.7860 (t, 2H, Ar-H) 7.6981-7.6828 (d, 2H, Ar-H), 7.6136-7.5166 (t, 3H, Ar-H), 7.4928 (s, 1H, thiazole proton), 7.4887 (s, 1H, imidazole proton) 7.1493 (s, 1H, imidazole proton), 4.5182 (s, 2H, -$CH_2$- proton), 2.2580 (s,3H, -$CH_3$ in imidazole). $^{13}$C NMR (DMSO) δ ppm: 167.9 (C=O), 150.0, 146.0, 142.8 (C=N), 138.8-16.1 (16Ar-C), 54.4 ($CH_2$-), 28.1 ($CH_3$). Mass (LC-MS): *m/z* 399.12 (M), 400.23 (M+1, 100%).

**5-Methyl-(4-methyl-1*H*-imidazole-1-yl)methyl)-3-((4-phenylthiazol-2-yl) imino)indolin-2-one (3b):** Yield: 69%, m.p.: 191-193 ºC. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3052 (C-H *str.*, Ar-H), 2930, 2823 (C-H *str.*, aliphatic –CH-), 2328-CSC- *str.*), 1722 (-C=O *str.,* isatin), 1628 (-=N *str.*), 1530 (C=CH *str.*), 1077 (-C-N *str.*).$^1$H NMR (DMSO) δ ppm: 8.1035 (s, 1H, Ar-H), 7.8867-7.8482 (d, 2H, Ar-H), 7.6828-7.6381 (t, 3H, Ar-H)), 7.5863-7.5534 (d, 2H, Ar-H) 7.5138 (s, 1H, thiazole proton), 7.1379 (s, 1H, imidazole proton), 7.1134 (s, 1H, imidazole proton), 4.4118 (s, 2H, -$CH_2$- proton), 2.1203 (s,3H, -$CH_3$ in imidazole), 1.9738 (s, 3H, -$CH_3$ in isatin). $^{13}$C NMR (DMSO) δ ppm: 167.6 (C=O), 151.3, 142.2, 139.5 (C=N), 136.8-125.8 (15Ar-C), 53.4 ($CH_2$-), 28.8, 24.3 ($CH_3$). Mass (LC-MS): *m/z* 413.13 (M), 414.11 (M+1, 100%).

**5-Chloro-1-((4-methyl-1*H*-imidazole-1-yl)methyl)-3-((4-phenylthiazol-2-yl)imino)indolin-2-one (3c):** Yield: 75%, m.p.: 213-215 ºC. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3020 (-CH *str.*), 2991,




Scheme-I

2875 (-CH *str.*, aliphatic –CH-), 2305 (-CSC- *str.*), 1702 (-C=O *str.*, isatin), 1603 (-C=N *str.*), 1523 (-C=CH *str.*), 1062 (-C-N *str.*), 798 (C-Cl *str.*).$^1$H NMR (DMSO) δ ppm: 8.3505 (s, 1H, Ar-H), 8.0631-8.0441 (d, 2H, Ar-H), 7.9452-7.9219 (t, 3H, Ar-H), 7.8287-7.8162 (d, 2H, Ar-H), 7.7783 (s, 1H, thiazole proton), 7.1831 (s, 1H, imidazole proton), 7.1610 (s, 1H, imidazole proton), 4.8135 (s, 2H, -$CH_2$- proton), 2.1307 (s,3H, -$CH_3$ in imidazole). $^{13}$C NMR (DMSO) δ ppm: 164.3 (C=O), 155.7, 146.24, 142.1 (C=N), 138.6-122.8 (14Ar-C), 53.($CH_2$-), 25.3, 23.1 ($CH_3$). Mass (LC-MS): *m/z* 433.08 (M), 434.29 (M+1, 100%), 434.06 (M+2, 30%).

**5-Chloro-1-((4-methyl-1*H*-imidazol-1-yl)methyl)-3-((4-(*p*-tolyl)thiazol-2-yl)imino)indolin-2-one (3d):** Yield: 78%, m.p.: 227-229 ºC. IR (KBr, $\nu_{max}$, cm$^{-1}$): 3090 (-CH *str.*, Ar-H), 2904, 2834 (-CH *str.*, aliphatic –CH-), 2331 (-CSC- *str.*), 1704 (-C=O *str.*, isatin), 1630 (-C=N *str.*), 1564 (-C=CH *str.*), 1056 (-C-N *str.*), 851 (-C-Cl *str.*).$^1$H NMR (DMSO δ ppm): 8.4763 (s, 1H, Ar-H), 7.9581 (d, 2H, Ar-H), 7.8812 (d, 2H, Ar-H), 7.8433 (d, 2H, Ar-H), 7.7941 (s, 1H, thiazole proton), 7.1474 (s, 1H, imidazole proton) 7.1348 (s, 1H, imidazole proton), 4.5813 (s, 2H, -$CH_2$- protons), 2.2408 (s,3H, -$CH_3$ in imidazole), 1.9054 (s, 3H, Ar-$CH_3$ protons). $^{13}$C NMR (DMSO δ ppm): 160.1 (C=O), 156.3, 145.7, 136.2 (C=N), 139.2-128.1 (14Ar-C), 58.42$CH_2$-), 29.1, 26.7, 23.5 (3$CH_3$) Mass (LC-MS): *m/z* 477.09 (M), 478.21 (M+1, 100%), 479.04 (M+2, 30%).

**1-((4-Methyl-1*H*-imidazol-1-yl)methyl)-5-nitro-3-((4-phenylthiazol-2-yl) imino)indolin-2-one (3e):** Yield: 73%, m.p.: 231-233 ºC. IR (KBr, $\nu_{max}$, cm$^{-1}$): 3030 (-CH *str.*, Ar-H), 2992, 2839 (-CH *str.*, aliph-CH-), 2350 (-CSC- *str.*), 1705 (-C=O *str.*, isatin), 1645 (-C-$NO_2$ *str.*), 1610 (-C=N *str.*), 1530 (-C=CH *str.*), 1043 (-C-N *str.*). $^1$H NMR (DMSO) δ ppm: 8.1541 (s, 1H, Ar-H), 7.8876-7.8363 (t, 3H, Ar-H), 7.7927-7.7710 (d, 2H, Ar-H), 7.6983-7.6729 (d, 2H, Ar-H), 7.5147 (s, 1H, thiazole proton), 7.4556 (s, 1H, imidazole proton), 7.4147 (s, 1H, imidazole proton), 4.3310 (s, 2H, -$CH_2$- proton), 2.2302 (s,3H, -$CH_3$ in imidazole). $^{13}$C NMR (DMSO) δ ppm: 165.3 (C=O), 154.2, 146.5, 137.2 (C=N), 136.2-120.7 (13Ar-C), 54.0 ($CH_2$-), 26.7, 24.1 (2$CH_3$) Mass (LC-MS): *m/z* 444.10 (M), 445.23 (M+1, 100%).

**1-((4-Methyl-1*H*-imidazole-1-yl)methyl)-5-nitro-3-((4-(*p*-tolyl)thiazol-2-yl) imino)indolin-2-one (3f):** Yield: 85%, m.p.: 151-153 ºC. IR (KBr, $\nu_{max}$, cm$^{-1}$): 3078 (-CH *str.*, Ar-H), 2965, 2804 (-CH *str.*, aliphatic -CH-), 2365 (-CSC- *str.*), 1712 (-C=O *str.*, isatin), 1656 (-$NO_2$, *str.,* Ar-$NO_2$), 1621 (-C=N *str.*), 1589 (-C=CH *str.*), 1045 (-C-N *str.*).$^1$H NMR (DMSO) δ ppm: 8.3622 (s, 1H, Ar-H), 7.8776-7.7654 (d, 2H, Ar-H), 7.6754-7.5763 (d, 2H, Ar-H), 7.3743-7.2943 (d, 2H, Ar-H), 7.1092 (s, 1H, thiazole proton), 7.0232 (s, 1H, imidazole proton), 6.9083 (s, 1H, imidazole proton), 4.6543 (s, 2H, -$CH_2$- proton), 2.3662 (s,3H, -$CH_3$ in imidazole), 2.0934 (s,3H, Ar-$CH_3$ protons). $^{13}$C NMR (DMSO) δ ppm: 168.9 (C=O), 155.9, 143.1, 137.3 (C=N), 133.5-123.1 (13Ar-C), 58.2 ($CH_2$-), 26.9, 23.1, 22.0 (3$CH_3$) Mass (LC-MS): *m/z* 458.12 (M), 459.28 (M+1, 100%).

**5-Methyl-1-((4-methyl-1*H*-imidazole-1-yl)methyl)-3-((4-(*p*-tolyl)thiazol-2-yl)imino)indole-2-one (3g):** Yield: 84%, m.p.: 239-241 ºC. IR (KBr, $\nu_{max}$, cm$^{-1}$): 3087 (-CH *str.*, Ar-H), 2954, 2890 (-CH, aliphatic -CH-), 2301 (-CSC- *str.*), 1721 (-C=O *str.*, isatin), 1623 (-C=N *str.*), 1576 (-C=CH *str.*), 1067 (-C-N *str.*). $^1$H NMR (DMSO) δ ppm: 8.2432 (s, 1H, Ar-H), 8.1872-8.0983 (d, 2H, Ar-H), 7.9872-7.8976 (d, 2H, Ar-H), 7.7683-7.6533 (d, 2H, Ar-H), 7.4532 (s, 1H, thiazole proton), 7.2893 (s, 1H, imidazole proton), 7.1093 (s, 1H, imidazole proton), 4.3523 (s, 2H, -$CH_2$- proton), 2.1983 (s,3H, -$CH_3$ in imidazole), 2.0453 (s, 3H, -$CH_3$ in isatin), 1.8973 (s,3H, Ar-$CH_3$ protons). $^{13}$C NMR (DMSO) δ ppm: 164.8 (C=O), 154.2, 148.3, 137.2 (C=N), 138.5-127.2 (13Ar-C), 55.8 ($CH_2$-), 26.3, 22.1 (4$CH_3$). Mass (LC-MS): *m/z* 427.15 (M), 428.45 (M+1, 100%).

**3-(4-(4-Chlorophenyl)thiazol-2-yl)imino)-5-methyl-1-((4-methyl-1*H*-imidazol-1-yl)methyl)indole-2-one (3h):** Yield: 69%, m.p.: 205-207 ºC. IR (KBr, $\nu_{max}$, cm$^{-1}$): 3056 (-CH *str.*, Ar-H), 2993, 2876 (-CH *str.*, aliphatic -CH-), 2355 (-CSC- *str.*), 1717 (-C=O *str.*, isatin), 1612 (-C=N *str.*), 1545 (-C=CH *str.*), 1098 (-C-N *str.*), 798 (-C-Cl *str.*). $^1$H NMR (DMSO) δ ppm: 8.3564 (s, 1H, Ar-H), 8.3421-8.2543 (d, 2H, Ar-H), 8.1232-8.0954 (d, 2H, Ar-H), 7.9861-7.8943 (d, 2H, Ar-H), 7.5564 (s, 1H, thiazole proton), 7.4032 (s, 1H, imidazole proton), 7.3123 (s, 1H, imidazole proton), 4.2133 (s, 2H, -$CH_2$- proton), 2.3452 (s, 3H, -$CH_3$ in imidazole), 2.0532 (s,3H, Ar-$CH_3$ protons). $^{13}$C NMR (DMSO) δ ppm: 163.0 (C=O), 156.2, 146.3, 138.2 (C=N), 135.1-128.2 (13Ar-C), 56.2 ($CH_2$-), 27.3, 22.7 (2$CH_3$) Mass (LC-MC): *m/z* 447.09 (M), 448.31 (M+1, 100%), 449.03 (M+2, 30%).

**5-Chloro-3-((4-(4-chlorophenyl)thiazol-2-yl)imino)-1-((4-methyl-1*H*-imidazol-1-yl)methyl)indole-2-one (3i):** Yield: 77%, m.p.: 217-219 ºC. IR (KBr, $\nu_{max}$, cm$^{-1}$): 3067 (-CH *str.*, Ar-H), 2987, 2856 (-CH *str.*, aliphatic -CH-), 2345 (-CSC- *str.*), 1721 (-C=O *str.*, isatin), 1621 (-C=N *str.*), 1542 (-C=CH *str.)*, 1023 (-C-N *str.*), 789 (-C-Cl *str.*).$^1$H NMR (DMSO) δ ppm: 8.2363 (s, 1H, Ar-H), 7.9872-7.8733 (d, 2H, Ar-H), 7.6754-7.5432 (d, 2H, Ar-H), 7.4763-7.4003 (d, 2H, Ar-H), 7.2342 (S, 1H, thiazole proton), 7.0232 (s, 1H, imidazole proton), 6.9802 (s, 1H, imidazole proton), 4.2432 (s, 2H, -$CH_2$- proton), 2.2031 (s,3H, -$CH_3$ in imidazole). $^{13}$C NMR (DMSO) δ ppm: 170.2 (C=O), 154.1,146.1, 142.2 (C=N), 138.1-123.1 (14Ar-C), 53.0 ($CH_2$-), 24.3, 20.1 (2$CH_3$). Mass (LC-MS) *m/z* 467.04 (M), 468.12 (M+1, 100%), 469.11 (M+2, 30%).

**5-Chloro-3-((4-(4-methoxyphenyl)thiazol-2-yl)imino)-1-((4-methyl-1*H*-imidazol-1-yl)methyl)indole-2-one (3j):** Yield: 85%, m.p.: 251-253 ºC. IR (KBr, $\nu_{max}$, cm$^{-1}$): 3078 (-CH *str.*, Ar-H), 2965, 2856 (-CH *str.*, aliphatic -CH-), 2356 (-CSC- *str.*), 1709 (-C=O *str.*, isatin), 1630 (-C=N *str.*), 1524 (-C=CH *str.*), 1087 (-C-N *str.*), 802 (-C-Cl *str.*).$^1$H NMR (DMSO) δ ppm: 8.5431 (s, 1H, Ar-H), 8.3724 (d, 2H, Ar-H), 8.1032 (d, 2H, Ar-H), 7.9421 (d, 2H, Ar-H), 7.5643 (S, 1H, thiazole proton), 7.4324 (s, 1H, imidazole proton), 7.2534 (s, 1H, imidazole proton), 4.6731 (s, 2H, -$CH_2$- proton), 3.5323 (s, 3H, Ar-$OCH_3$ protons), 2.3452 (s,3H, -$CH_3$ in imidazole), 2.0942 (s,3H, Ar-$CH_3$ protons). $^{13}$C NMR (DMSO) δ ppm: 168.3 (C=O), 157.3, 145.3, 140.3 (C=N), 139.5-124.5 (14Ar-C), 63.4 ($OCH_3$), 56.2 ($CH_2$-), 23.1, 22.3 (2$CH_3$) Mass (LC-MS): *m/z* 443.14 (M), 444.19 (M+1, 100%).

**3-((4-(4-Chlorophenyl) thiazol-2-yl)imino)-1-((4-methyl-1*H*-imidazole-1-yl) methyl)-5-nitroindole-2-one**

**(3k):** Yield: 76%, m.p.: 219-221 ºC. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3087 (-CH *str.,* Ar-H), 2967, 2898 (-CH *str.*, aliphatic -CH-), 2367 (-CSC- *str.*), 1711 (-C=O *str.*, isatin), 1643 (-$NO_2$ *str.*, Ar-$NO_2$),1646 (-C=N *str.*), 1523 (-C=CH *str.*), 1043 (C-N *str.*), 795 (-C-Cl).$^1$H NMR (DMSO) δ ppm: 8.4892 (s, 1H, Ar-H), 8.0932 (d, 2H, Ar-H), 7.9832 (d, 2H, Ar-H), 7.8094 (d, 2H, Ar-H), 7.6632 (s, 1H, thiazole proton), 7.5433 (s, 1H, imidazole proton), 7.3432 (s, 1H, imidazole proton), 4.2893 (s, 2H, -$CH_2$- proton), 2.4533 (s, 3H, -$CH_3$ in imidazole). $^{13}$C NMR (DMSO) δ ppm: 165.3 (C=O), 153.7,145.8,140.4 (C=N), 139.4-125.2 (14Ar-C), 56.2 ($CH_2$-), 27.0, 23.9 ($2CH_3$). Mass (LC-MS): *m/z* 478.06 (M), 479.43 (M+1, 100%), 480.37 (M+2, 30%).

**3-((4-(4-Methoxyphenyl)thiazol-2-yl)imino)-1-((methyl-1*H*-imidazol-1-yl)methyl)-5-nitroindolin-2-one (3l):** Yield: 81%, m.p.: >270 ºC. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3067 (-CH *str.*, Ar-H), 2998, 2878 (-CH *str.*, aliphatic -CH-), 2321 (-CSC- *str.*), 1710 (-C=O *str.*, isatin), 1643 (-$NO_2$ *str.*, Ar-$NO_2$), 1616 (-C=N *str.*), 1532 (C=CH *str.*), 1087 (C-N).$^1$H NMR (DMSO) δ ppm: 8.3897 (s, 1H, Ar-H), 8.2543-8.2093 (d, 2H, Ar-H), 7.9890-7.8943 (d, 2H, Ar-H), 7.6875-7.5908 (d, 2H, Ar-H), 7.4563 (s, 1H, thiazole proton), 7.2834 (s, 1H, imidazole proton), 6.9981 (s, 1H, imidazole proton), 4.6782 (s, 2H, -$CH_2$- proton), 3.5723 (s, 3H,Ar-$OCH_3$ protons), 2.3212 (s,3H, -$CH_3$ in imidazole). $^{13}$C NMR (DMSO) δ ppm: 171.5 (C=O), 159.3, 145.2, 141.6 (C=N), 137.4-121.9 (14Ar-C), 63.4 ($OCH_3$), 51.5 ($CH_2$-), 28.4, 25.6 ($2CH_3$). Mass (LC-MS): *m/z* 474.11 (M), 475.15 (M+1, 100%).

### Pharmacological activity

**Anticancer activity:** All the newly synthesized thiazole, imidazole-indole hybrids were tested for *in vitro* anticancer activity against two cell lines like MCF-7 and $SKVO_3$ with doxorubicin as standard drug. The cell viability was carried out by MTT assay method with three independent experiments *via* six concentrations of synthesized compounds in triplicates [13]. Every cells were tripsinized and perform the trypan blue assay to know viable cells in cell suspension. Then cell viable were counted by haemocytometer and seeded at density of $5.0 \times 10^3$ cells/well in 100 µL media in 96 well plate culture medium. Finally, all the plates were incubated overnight at 37 ºC. After the incubation period, take the old media and add some fresh media 100 µL with different concentrations of test compounds in labelled wells in 96 plates. After 48 h, discard the drug solution and add fresh media with MTT solution (0.5 mg/mL) to each plates. All well plates were incubated at 37 ºC for 3 h. After completion of the incubation period, the precipitates were formed *via* the reduction of the MTT salt to chromophore formosan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition and viability was calculated using standard formula and concentration of test needed to inhibit cell growth by 50% value. The $IC_{50}$ values were generated from the dose response curve for each cell line by origin software.

**Antibacterial activity:** All the synthesized compounds (**3a-l**) were screened for antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella paratyphi* using cup-plate agar diffusion method (**3a-l**) by measuring the zone inhibition in mm. The streptomycin (50 µg/mL) used as a standard drug.

**Molecular docking studies:** In drug design process, the molecular docking models were applied to investigate the binding mode of target molecules *via* selected proteins with PDB ID for cancer cell lines. The synthesized ligands were docked in to the active site of the EGFR protein using Ligprep tool of Schrödinger suite and consequently to rationalize the obtained biological data. The protein-ligand interactions of the dataset ligands were observed by using structurally optimized protein shape with Glide Xp docking protocol. Initially, a 3D grid used to be set up to the binding active site (pocket) of the EGFR protein into all the dataset ligands had been docked. Finally, the binding interactions and it was calculated in phrases of Glide score. It is a combination of hydrophilic, hydrophobic, metal binding groups, van der Waals energy, freezing rotatable bonds and polar interactions with receptor. Highest docked pose with lowest glide score was recorded for each ligand and extra precision was performed by using Schrödinger suite [14].

## RESULTS AND DISCUSSION

A series of synthesized novel thiazole, imidazole-indole hybrids (**3a-l**) were confirmed through spectral analysis like FT-IR, $^1$H NMR and mass spectroscopies. Microwave-assisted synthesis was used to synthesize **1a-g** compounds *via* a cyclization mechanism involving substituted acetophenone and thiourea, which further undergone the Schiff base mechanism to yield isatin Schiff bases (**2a-g**). Finally, it was reacted with formaldehyde and 4-methyl imidazole by Mannich base mechanism to form the target molecules (**3a-l**).

In the IR spectra of novel hybrid compounds, the carbonyl stretching (>C=O) bands were observed at between 1728-1695 $cm^{-1}$. All the synthesized compounds contain aromatic and aliphatic C-H stretching were observed around observed at around 3098-3005 $cm^{-1}$ and 2934-2765 $cm^{-1}$, repectively. Few compounds containing Ar-Cl group showed a strong absorption peak around at 810-780 $cm^{-1}$. The $^1$H NMR spectrum of all compounds showed that singlet protons at δ 2.01-2.45 ppm due to -$CH_3$ protons of imidazole ring and at δ 4.25-4.73 ppm due to methylene protons (-$CH_2$-). All the compounds were showed singlets, doublets and triplets at δ 6.83-8.52 ppm due to the presence aromatic protons. The $^1$H NMR spectrum of compound **3j** and **3j** showed the singlets within the region at δ 3.78-3.52 ppm due to methoxy protons (-$OCH_3$). The remaining derivatives also exhibited satisfactory chemical shift values (δ), which confirmed the derived structure. In $^{13}$C NMR of the synthesized hybrid compounds, the peak appeared at δ 159-141 ppm were conformed by the presence of carbonyl carbon (C=O), whereas the imine carbons (C=N) were conformed at δ 159-141 ppm. The presence of a signal at δ 20-29 ppm was confirmed by methyl carbon (-$CH_3$) for all of the derivatives, moreover, the signal at δ 50-58 ppm shows the methine linkage (-$CH_2$). The mass spectrum of all derivatives (**3a-l**) were also conformed by their molecular ion peak and molecular weight.

**Anticancer activity:** The newly synthesized thiazole, imidazole-indole hybrids (**3a-l**) were tested for anticancer activity by MTT assay method *via* two cancer cell line's (MCF-7 and SKVO3). The results were compared with the standard doxorubicin drug. The anticancer screening results of novel thiazole, imidazole-indole hybrids were expressed as $IC_{50}$ and the values are summarized in Table-1. It was observed that the $IC_{50}$ values were in the range of 24.11 to 56.57 µg against MCF-7 Cell lines and 36.12 to 76.03 µg against SKVO3 cell lines. Compound **3i** ($IC_{50}$ value of 26.98 µg, 36.12 µg) and **3j** ($IC_{50}$ value of 24.11 µg, 38.43 µg) exhibited the good anticancer activity against both cell lines, while the remaining compounds showed the moderated activity.

TABLE-1
ANTICANCER ACTIVITY OF NOVEL THIAZOLE, IMIDAZOLE-INDOLE HYBRIDS ON MCF-7 AND SKVO3 CELL LINES

| Sample description | Test parameters $IC_{50}$ (µg) | |
|---|---|---|
| | MCF-7 | SKVO3 |
| **3b** | 45.76 ± 0.512 | 67.23 ± 0.211 |
| **3d** | 56.57 ± 0.051 | 41.45 ± 0.032 |
| **3e** | 53.78 ± 0.031 | 63.12 ± 0.093 |
| **3f** | 35.97 ± 0.082 | 76.03 ± 0.154 |
| **3i** | 26.98 ± 0.203 | 36.12 ± 0.221 |
| **3j** | 24.11 ± 0.172 | 38.43 ± 0.630 |
| **3k** | 49.03 ± 0.451 | 73.13 ± 0.165 |
| Doxorubicin | 13.12 ± 0.391 | 16.01 ± 0.893 |

**Antibacterial activity:** The novel thiazole, imidazole-indole hybrids were also screened for antibacterial activity using agar diffusion method. From the results, compounds **3d** (23 mm), **3f** (25 mm), **3j** (24 mm) against *S. aureus*; Compound **3c** (23 mm), **3d** (20 mm) against *B. subtilis*; compound **3c** (22 mm), **3f** (23 mm), **3j** (23 mm) against *E. coli*; compound **3d** (25 mm) against *S. paratyphi* shows almost similar activity as with standard drug (streptomycin) (Table-2).

**Molecular docking:** Molecular docking studies was carried out to analyzed compounds binding mode against breast and ovaries cancer cell lines. The ligand interactions showed the good resolution in the compound binding site and standard drugs in the active site of PDB Id: 1M17. Molecular docking research carried out by using the Ligprep tool of Schrödinger suite and the Glide dock score of the dataset ligands along with the interaction amino acids. The results with EGFR protein, compound **3d** reported the highest docking score of -6.538 with glide binding energy of -44.297 Kcal/mol (Table-3). The docking scores of the all target molecules were ranged from -6.538 (compound **3d**) to -5.125 (compound **3f**). Except compound **3b**, all compounds had one hydrogen bond with MET 769 amino acid. Compounds **3i** and **3f** had three salt bridges each with LYS 721, GLU 738 and ASP 831 amnio acids with $–NO_2$ group (Fig. 1). Solvent exposure regions were observed for com-pounds **3d** (4-chlorophenyl nucleus and 4-methyl-1*H*-imidazole nucleus), **3f** (4-(*p*-tolyl) thiazole scaffold), **3h** (4-(4-chlorophenyl)thiazole scaffold), **3i** (4-(4-methoxyphenyl)-thiazole scaffold) and **3j** (4-(4-methoxyphenyl)thiazole scaffold).

## Conclusion

A series of novel thiazole, imidazole-indole hybrids (**3a-1**) were synthesized by microwave method *via* Schiff and Mannich

TABLE-2
ANTIBACTERIAL ACTIVITY OF NOVEL THIAZOLE, IMIDAZOLE-INDOLE HYBRIDS (**3a-l**)

| Compound | R | $R_1$ | m.f. | Zone of inhibition (mm) | | | |
|---|---|---|---|---|---|---|---|
| | | | | *S. aureus* | *B. subtilis* | *E. coli* | *S. paratyphi* |
| **3a** | H | H | $C_{22}H_{17}N_5OS$ | 13 | 11 | 15 | 19 |
| **3b** | H | $CH_3$ | $C_{23}H_{19}N_5OS$ | 10 | 13 | 09 | 10 |
| **3c** | H | Cl | $C_{22}H_{16}N_5ClOS$ | 13 | 23* | 22* | 15 |
| **3d** | $CH_3$ | Cl | $C_{23}H_{18}N_5OSCl$ | 23* | 20* | 19 | 25* |
| **3e** | H | $NO_2$ | $C_{22}H_{16}N_6O_3S$ | 12 | 14 | 10 | 161 |
| **3f** | $CH_3$ | $NO_2$ | $C_{23}H_{18}N_6O_3S$ | 25* | 16 | 23* | 14 |
| **3g** | $CH_3$ | $CH_3$ | $C_{24}H_{21}N_5OS$ | 13 | 15 | 19 | 10 |
| **3h** | Cl | $CH_3$ | $C_{23}H_{18}N_5OSCl$ | 14 | 09 | 14 | 17 |
| **3i** | Cl | Cl | $C_{22}H_{15}N_5Cl_2OS$ | 17 | 09 | 20 | 16 |
| **3j** | $OCH_3$ | $CH_3$ | $C_{24}H_{21}N_5O_2S$ | 24* | 15 | 23* | 10 |
| **3k** | Cl | $NO_2$ | $C_{22}H_{15}N_6ClO_3S$ | 16 | 09 | 18 | 15 |
| **3l** | $OCH_3$ | $NO_2$ | $C_{23}H_{18}N_6O_4S$ | 09 | 18 | 09 | 13 |
| Streptomycin | | | | 29 | 32 | 30 | 31 |

All values are expressed as % Inhibition; Bore size = 6 mm, Concentration of test compounds is 100 µg/mL

TABLE-3
*In silico* EGFR INHIBITION OF NOVEL THIAZOLE, IMIDAZOLE-INDOLE HYBRIDS (**3a-l**)-GLIDE DOCK SCORE OF THE DATASET LIGANDS

| Compd. No. | Dock score XP Gscore | No. of H-bonds | Interacting amino acids | H-bond lengths (Å) | Emodel energy | Glide energy |
|---|---|---|---|---|---|---|
| **3d** | -6.538 | 0 | – | – | -55.124 | -44.297 |
| **3j** | -6.190 | 1 | MET 769 | 1.97 | -64.076 | -47.620 |
| **3i** | -5.713 | 1 | MET 769 | 2.15 | -52.379 | -48.841 |
| **3h** | -5.547 | 1 | MET 769 | 2.23 | -58.157 | -46.578 |
| **3f** | -5.125 | 1 | MET 769 | 2.21 | -64.019 | -47.776 |

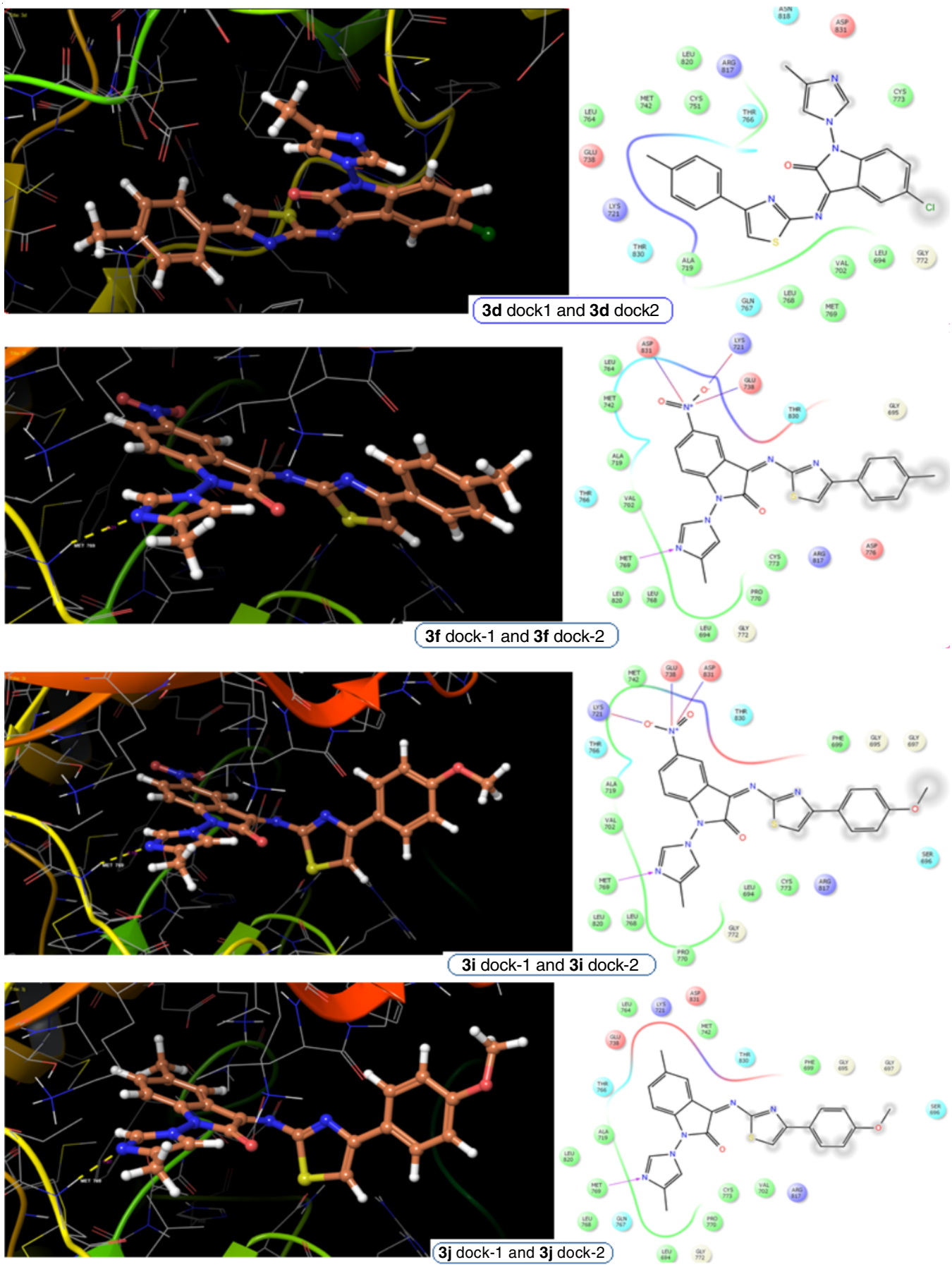



Fig. 1. Docking pose between the ligand and the protein (Dock1 and Dock2)-Compound **3d**, **3f**, **3i** and **3j**

base mechanism and characterized by spectral analysis. The present study showed that the anticancer and antibacterial activity of newly synthesized compound (**3a-l**) may altered due to the introduction of specific groups.

## ACKNOWLEDGEMENTS

The authors are thankful to The Director, CSIR–Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad, India for providing the spectral analysis data and GITAM School of Pharmacy, Hyderabad, India.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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