Asian Journal of Chemistry; Vol. 33, No. 9 (2021), 2027-2032

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23271

Synthesis, Characterization and Anticancer Activity of (5,1-Substituted)-3-(indoline-4-(thiophene-2-yl-methylene)-2-(*p*-tolyl)-2-methylene)-4,3-dihydro-1*H*-imidazole-5-one Derivatives

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Received: 26 April 2021; *Accepted*: 11 June 2021; *Published online*: 20 August 2021; AJC-20459

The synthesis of novel imidazole-5-one derivatives (**5a-j**) was allowed in a conventional method by way of Erlenmeyer and Schiff base mechanism. Compound **2a** was synthesized by Erlenmeyer reaction of *N*-(4-methoxy benzoyl)glycine with 2-thiophene-carboxaldehyde in the presence of acetic anhydride and anhydrous sodium acetate. Finally, it undergoes dehydration reaction with Schiff bases of isatin derivatives (**4a-j**) to yield final compounds **5a-j**. The organic potentials of the newly synthesized imidazole-5-one derivatives have been evaluated for their *in vitro* anticancer activity by MTT assay method. It against MCF-7 cells as comparison with doxorubicin popular drug. The synthesized compounds **5e**, **5f** and **5j** exhibited excellent anticancer activity against MCF-7 cell lines.

Keywords: Isatin derivatives, 4-Methoxy benzoyl glycine, Imidazole-5-one derivatives, Anticancer activity, MCF-7 cells.

INTRODUCTION

Imidazole is a category of heterocyclic compound that benefit one-of-a-kind interests due to the fact it belongs to a group of substances with activity in medicinal chemistry [1,2]. This tricyclic nucleus consequent is associated with anti-inflammatory [3,4], anticancer [5], antimicrobial [6], antitubercular [7,8], antiparasitic [9], antimalarial [10,11], antiviral [12,13] and fungicidal activities [14].

The synthesis of imidazole and its derivatives has attracted considerable attention from natural and medicinal chemists for many years, as a number of natural sources have been communicated to have this heterocyclic nucleus. Chemically, imidazole is additionally recognized *via* the names of 1,3-diazacyclopenta-2,4-diene. Because of the statistics cited above and the wide functions of imidazole molecule and its derivatives in medicinal chemistry an endeavor has been framed to synthesize novel (5,1-substituted)-3-(indoline-4-(thiophene-2-yl-methylene)-2-(*p*-tolyl)-2-methylene)-4,3-dihydro-1*H*-imidazole-5-one derivatives moiety as new anticancer agents.

EXPERIMENTAL

All the chemicals and reagents used in this work were of analytical grade and procured from the reputed commercial sources.

Synthesis of 4-methoxy benzoyl glycine derivatives (1a): Put together 10% of NaOH and 0.03 mol of glycine in a 250 mL conical flask. Further, added 0.03 mol of 4-methoxy benzoyl chloride in 5 parts to a solution. Stopper the vessel and shake aggressively after each addition till all the chloride has reacted. Then, the solutions were transferred to a beaker and sluice the conical flask with a little water. Placed little pieces of crushed ice to a solution. Added steadily 5 mL of HCl with stirring prior to the mixture. Finally, collected the crystalline precipitate of 4-methoxybenzoyl glycine on the Büchner funnel and air dried [15].

Synthesis of 2-(4-methoxy)phenyl-4-(thiophen-2-yl-methylene)-5-oxazolinone (2a): An equimolar concentration solution of thiophene-2-carboxaldehyde (0.01 mol), *N*-(4-methoxy benzoyl)glycine (0.01 mol) had been placed in a 250 mL conical flask. Further, added 2.85 mL (0.03 mol) of acetic anhydride, 0.82 g (0.01 mol) of anhydrous sodium acetate and heated on an electric hot plate with steady shaking. As quickly as mixture become clear, placed the flask into a warm water for 2 h. Cooled and then added 20 mL of ethanol slowly to the contents of flask. The reaction mixture was left for overnight, filtered the crystalline product with suction, washed with 5 mL of frozen alcohol and then subsequently washed with 5 mL of boiling water, dried at 100 ºC [16].

Synthesis of *N*-substituted isatin derivatives (3a-j): A 250 mL flask geared up with a magnetic stirring bar used to be charged with 100 mL of DMF solution and 13 mmol of NaOH. The mixtures were once stirred at room temperature for 5 min and added 10 mmol of isatin with constant stirring for 45 min. Further, added 11 mmol of alkyl halide and the stirring was continued for 12 h at 80 ºC. Finally, the mixture was diluted with 200 mL of water, extracted with ethyl acetate and dried over anhydrous sodium sulfate. Then, excess amount of solvent was discarded under vacuum and the residue was purified by column chromatography on silica gel using ethyl acetate and hexane (1:9). Finally, pure *N*-substituted isatin (**3a-j**) was obtained [17].

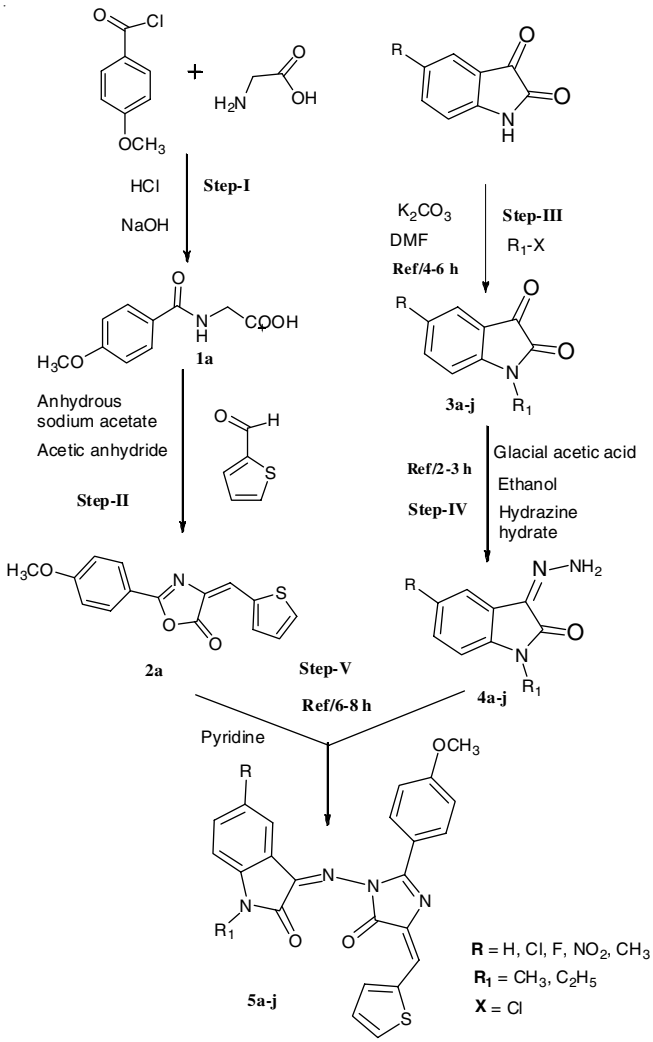
Synthesis of isatin Schiff's base derivatives (4a-j): A mixture of equimolar quantity of *N*-substituted isatin derivatives (**3a-j**) (0.01 mol) and hydrazine hydrate (0.01 mol) was dissolved in 20 mL of ethanol, refluxed for 2-3 h in the presence of few drops of glacial acetic acid. The progress of the reaction was monitored *via* TLC (*n*-hexane:EtOAc 7:3). The reaction mixture was once cooled to room temperature and stored in a fridge for 24 h. Finally, a solid was obtained, filtered off and recrystallized from methanol or ethanol to yield the crystalline solid [18] (**Scheme-I**).

The synthesized imidazole-5-one derivatives were screened for anticancer activities. Fourier Transform IR spectrometer (model Shimadzu 8700) in the range of 4000-400 cm^{-1} using KBr pellets. ^1H NMR spectra had been recorded on DPX-200 MHz NMR spectrometer using DMSO-d_6 and chemical shifts (δ) are delineated in ppm downfield from internal reference tetramethylsilane (TMS). Mass spectra were recorded on Mass spectrophotometer (Shimadzu) through LC-MS. Precoated silica gel G plates had been used to reveal the progress of reaction as well as to look at the purity of the compounds *n*-hexane: ethyl acetate (8:2).

Synthesis of 4-thiophen-2-ylmethylene-2-phenyl-1-(5-substituted-1-alkyl- indolin-2-one)-1*H*-imidazol-5(4*H*)-one (5a-j): A series of novel 4-thiophen-2-ylmethylene-2-phenyl-1- (5-substituted-1-alkyl-indolin-2-one)-1*H*-imidazol-5(4*H*)-one (**5a-j**) was synthesized by the reaction of equimolar (0.01 mol) portion of isatin Schiff's base (**4a-j**) and 2-(4-methoxy)phenyl-4-(thiophen-2-ylmethylene)-5-oxazolinone (0.01 mol) **2a** was refluxed in pyridine for 6-8 h. The development of the response was once monitored through TLC. After completion of the reaction ensuring mass used to be poured into crushed ice and neutralized with dil. HCl. The resulting precipitate was filtered, dried and recrystallized from methanol [1] (**Scheme-I**).

3-Hydrazineylidene-1-methyl-indolin-2-one (4a): Yield: 74%, m.p.: 143-145 ºC; m.f.: $C_9H_9N_3O$, IR (KBr, ν_{max}, cm^{-1}): 3309 (N-H *str.*, -NH_2), 3043 (C-H *str.*, Ar), 2927 (C-H *str.*, aliph.), 1712 (C=O *str.*, indole), 1605 (C=N, *str.*), 1524 (C=CH *str.*). ^1H NMR (DMSO) δ ppm: 8.2911-8.1897 (d, 2H, Ar-H), 7.9902-7.8943 (t, 2H, Ar-H), 4.5323 (s, 2H, -NH_2), 2.043 (s, 3H, N-CH_3). Mass (LC-MS): *m/z* 175 (M), 176 (M + 1, 100%).

3-Hydrazineylidene-1-methyl-5-methylindolin-2-one (4b): Yield: 78%, m.p.: 201-203 ºC; m.f.: $C_{10}H_{11}N_3O$, IR (KBr, ν_{max}, cm^{-1}): 3402 (N-H *str.*, -NH_2), 3001 (C-H *str.*, Ar), 2987 (C–H *str.*, aliph.), 1700 (C=O *str.*, indole), 1621 (C=N, *str.*) 1523 (C=CH *str.*). ^1H NMR (DMSO) δ ppm: 8.0982 (s, 1H, Ar-H), 7.8943-7.8903 (d, 2H, Ar-H), 4.3873 (s, 2H, -NH_2), 2.0234 (s, 3H, -CH_3). Mass (LC-MS): *m/z* 189 (M), 190 (M + 1, 100%).



Scheme-I: Synthetic route of (5,1-substituted)-3-(indoline-4-(thiophene-2-yl-methylene)-2-(*p*-tolyl)-2-methylene)-4,3-dihydro-1*H*-imidazole-5-one derivatives

3-Hydrazineylidene-1-methyl-5-chloroindolin-2-one (4c): Yield: 76%, m.p.: 187-189 ºC; m.f.: $C_9H_8N_3OCl$, IR (KBr, ν_{max}, cm^{-1}): 3387 (N-H *str.*, -NH_2), 3003 (C-H *str.*, Ar), 2967, (C-H *str.*, aliph.), 1723 (C=O *str.*, indole), 1612 (C=N, *str.*), 1532 (C=CH *str.*). ^1H NMR (DMSO) δ ppm: 8.0342 (s, 1H, Ar-H), 7.7893-7.6894 (d, 2H, Ar-H), 4.5673 (s, 2H, -NH_2), 2.2433 (s, 3H, N-CH_3). Mass (LC-MS): *m/z* 209 (M), 210 (M + 1, 100%).

3-Hydrazineylidene-1-methyl-5-nitroindolin-2-one (4d): Yield: 69%, m.p.: 188-190 ºC; m.f.: $C_9H_8N_4O_3$. IR (KBr, ν_{max}, cm^{-1}): 3327 (N-H *str.*, -NH_2), 3059 (C-H *str.*, Ar), 2936, (C-H *str.*, aliph.), 1703 (C=O *str.*, indole), 1616 (-NO_2, *str.*) 1536 (C=CH *str.*). ^1H NMR (DMSO) δ ppm: 8.3702 (s, 1H, Ar-H), 7.9508-7.7485 (d, 2H, Ar-H), 4.6706 (s, 2H, -NH_2), 2.0573 (s, 3H, -CH_3). Mass (LC-MS): *m/z* 220 (M), 221 (M + 1, 100%), 222 (M + 2, 30%).

3-Hydrazineylidene-1-methyl-5-fluoroindolin-2-one (4d): : Yield: 80%, m.p.: 203-205 °C; m.f.: $C_9H_8N_3O_3F$, IR

(KBr, ν_{max}, cm^{-1}): 3403 (N-H *str.*, -NH_2), 3021 (C-H *str.*, Ar), 2954 (C-H *str.*, aliph.), 1715 (C=O *str.*, indole), 1621 (C=N, *str.*), 1527 (C=CH *str.*). ^{1}H NMR (DMSO) δ ppm: 8.1243 (s, 1H, Ar-H), 7.9832-7.8934 (d, 2H, Ar-H), 4.5019 (s, 2H, -NH_2), 2.1823 (s, 3H, N-CH_3). Mass (LC-MS): *m/z* 203 (M), 204 (M + 1, 100%), 205 (M + 2, 30%).

3-Hydrazineylidene-1-ethyl-indolin-2-one (4f): Yield: 78%, m.p.: 144-146 ºC; m.f.: $C_{10}H_{11}N_3O$, IR (KBr, ν_{max}, cm^{-1}): 3420 (N-H *str.*, -NH_2), 3020 (C-H *str.*, Ar), 2987, 2894 (C-H *str.*, aliph.), 1716 (C=O *str.*, indole), 1603 (C=NO, *str.*) 1543 (C=CH *str.*). ^{1}H NMR (DMSO) δ ppm: 8.0324-8.0021 (d, 2H, Ar-H), 7.8342-7.8021 (d, 2H, Ar-H), 4.3448 (s, 2H, -NH_2), 2.1240 (q, 2H, N-CH_2), 1.9908 (t, 3H, -CH_3). Mass (LC-MS): *m/z* 189 (M), 190 (M + 1, 100%).

3-Hydrazineylidene-1-methyl-5-nitroindolin-2-one (4g): Yield: 69%, m.p.: 188-190 ºC; m.f.: $C_9H_8N_4O_3$, IR (KBr, ν_{max}, cm^{-1}): 3327 (N-H *str.*, -NH_2), 3059 (C-H *str.*, Ar), 2936, (C–H *str.*, aliph.), 1703 (C=O *str.*, indole), 1616 (-NO_2, *str.*) 1536 (C=CH *str.*). ^{1}H NMR (DMSO) δ ppm: 8.3702 (s, 1H, Ar-H), 7.9508-7.7485 (d, 2H, Ar-H), 2.0573 (q, 2H, N-CH_2), 1.9280 (t, 3H, -CH_3). Mass (LC-MS): *m/z* 220 (M), 221 (M + 1, 100%).

3-Hydrazineylidene-1-ethyl-5-chloroindolin-2-one (4h): Yield: 82%; m.p.: 163-165 ºC; m.f.: $C_{10}H_{10}N_3OCl$, IR (KBr, ν_{max}, cm^{-1}): 3403 (N-H *str.*, -NH_2), 3002 (C-H *str.*, Ar), 2976, 2890 (C-H *str.*, aliph.), 1718 (C=O *str.*, indole), 1603 (C=N, *str.*), 1512 (C=CH *str.*). ^{1}H NMR (DMSO) δ ppm: 8.2742 (s, 1H, Ar-H), 7.8983-7.8023 (d, 2H, Ar-H), 4.5021 (s, H, -NH_2), 2.1095 (q, 2H, N-CH_2), 1.7892 (t, 3H, -CH_3). Mass (LC-MS): *m/z* 223 (M), 224 (M + 1, 100%), 225 (M + 2, 30%).

3-Hydrazineylidene-1-ethyl-5-fluoroindolin-2-one (4i): Yield: 76%; m.p.: 129-131 ºC; m.f.: $C_{10}H_{10}N_3OF$, IR (KBr, ν_{max}, cm^{-1}): 3373 (N-H *str.*, -NH_2), 3020 (C-H *str.*, Ar), 2985 (C-H *str.*, aliph.), 1700 (C=O *str.*, indole), 1637 (C=N, *str.*) 1549 (C=CH *str.*). ^{1}H NMR (DMSO) δ ppm: 7.7837 (s, 1H, Ar-H), 7.6933-7.6808 (d, 2H, Ar-H), 4.5491 (s, 2H, -NH_2), 2.8060 (q, 2H, N-CH_2), 1.9280 (t, 3H, -CH_3). Mass (LC-MS): *m/z* 234 (M), 235 (M + 1, 100%), 236 (M + 2, 30%).

3-Hydrazineylidene-1-ethyl-5-methylindolin-2-one (4j): Yield: 77%, m.p.: 141-143 ºC; m.f.: $C_{10}H_{13}N_3O$, IR (KBr, ν_{max}, cm^{-1}): 3420 (N-H *str.*, -NH_2), 3023 (C-H *str.*, Ar), 2923, 2897 (C–H *str.*, aliph.), 1710 (C=O *str.*, indole), 1603 (C=N, *str.*), 1525 (C=CH *str.*). ^{1}H NMR (DMSO) δ ppm: 8.1203 (s, 1H, Ar-H), 7.8808-7.8021 (d, 2H, Ar-H), 4.5012 (s, 2H, -NH_2), 2.2121 (q, 2H, N-CH_2), 2.0342 (s, 3H, Ar-CH_3), 1.9002 (t, 3H, -CH_2-CH_3). Mass (LC-MS): *m/z* 203 (M), 204 (M + 1, 100%).

4-Thiophen-2-ylmethylene-2-(4-methoxy)phenyl-1-(*N*-methyl-indolin-2-one)-1*H*-imidazol-5(4*H*)-one (5a): Yield: 82%, m.p.: 235-237 ºC; *m.w.*: 442.8 g/mol; m.f.: $C_{24}H_{18}N_4O_3S$, R_f: 0.67. IR (KBr, ν_{max}, cm^{-1}): 3060 (C-H *str.*, Ar), 2934, 2821, 2719 (C–H *str.*, aliph.), 2365 (C-S-C *str.*, thiophene), 1766 (C=O *str.*, indole), 1709 (C=O *str.*, imidazole), 1605 (C=N, *str.*) 1515 (C=CH *str.*), 1446 (C=C *str.*, Ar). ^{1}H NMR (DMSO) δ ppm: 8.893 (s, 1H, =CH-Ar), 7.979-7.805 (d, 2H, Ar-H), 7.687-7.633 (d, 2H, Ar-H), 7.538-7.522 (d, 2H, Ar-H), 7.489-7.441 (t, 2H, Ar-H), 7.393-7.383 (d, 2H, Ar-H), 7.438 (t, 1H, Ar-H), 3.861 (s, 3H, -OCH_3), 3.163 (s, 3H, N-CH_3). ^{13}C NMR (DMSO) δ ppm: 179, 178 (2C, CO), 169, 157 (2C, C=N), 153-106 (18C, Ar-C), 54 (1C, -OCH_3), 27 (1C, N-CH_3). Mass (LC-MS): *m/z* 442 (M), 443 (M + 1, 100%).

4-Thiophen-2-ylmethylene-2-(4-methoxy)phenyl-1-(5-methyl-*N*-methyl-indolin-2-one)-1*H*-imidazol-5(4*H*)-one (5b): Yield: 76%, m.p.: 223-225 ºC; *m.w.*: 456.1 g/mol, m.f.: $C_{25}H_{20}N_4O_3S$, R_f: 0.61. IR (KBr, ν_{max}, cm^{-1}): 3009 (C-H *str.*, Ar), 2958, 2848, 2730 (C-H *str.*, aliph.), 2393 (C-S-C *str.*, thiophene), 1730 (C=O *str.*, indole), 1707 (C=O *str.*, imidazole), 1613 (C=N, *str.*) 1511 (C=CH *str.*), 1437 (C=C *str.*, Ar). ^{1}H NMR (DMSO) δ ppm: 8.862 (s, 1H, =CH-Ar), 8.107 (s, 1H, Ar-H), 7.881-7.848 (d, 2H, Ar-H), 7.688-7.636 (d, 2H, Ar-H), 7.581-7.578 (t, 2H, Ar-H), 7.558-7.513 (d, 2H, Ar-H), 7.147-7.115 (t, 1H, Ar-H), 3.705 (s, 3H, -OCH_3), 3.063 (s, 3H, N-CH_3), 2.018 (s, 3H, -CH_3). ^{13}C NMR (DMSO) δ ppm: 185, 180 (2C, CO), 167, 161 (2C, C=N), 151-127 (18C, Ar-C), 50 (1C, -OCH_3), 28 (1C, N-CH_3), 23 (1C, Ar-CH_3). Mass (LC-MS): *m/z* 456 (M), 457 (M + 1, 100%).

4-Thiophen-2-ylmethylene-2-(4-methoxy)phenyl-1-(5-chloro-*N*-methyl-indolin-2-one)-1*H*-imidazol-5(4*H*)-one (5c): Yield: 68%, m.p.: 257-259 ºC; *m.w.*: 476.2 g/mol, m.f.: $C_{24}H_{17}N_4O_3SCl$, R_f: 0.56. IR (KBr, ν_{max}, cm^{-1}): 3050 (C-H *str.*, Ar), 2953, 2835, 2709 (C–H *str.*, aliph.), 2380 (C-S-C *str.*, thiophene), 1737 (C=O *str.*, indole), 1709 (C=O *str.*, imidazole), 1626 (C=N, *str.*) 1537 (C=CH *str.*), 1435 (C=C *str.*, Ar). ^{1}H NMR (DMSO) δ ppm: 8.558 (s, 1H, =CH-Ar), 8.308-8.306 (d, 2H, Ar-H), 8.153 (s, 1H, Ar-H), 8.119-8.099 (d, 2H, Ar-H), 8.058-8.018 (d, 2H, Ar-H), 7.947-7.848 (t, 1H, Ar-H), 7.809-7.090 (d, 2H, Ar-H), 3.614 (s, 3H, -OCH_3), 3.031 (s, 3H, N-CH_3). ^{13}C NMR (DMSO) δ ppm: 179, 178 (2C, CO); 165, 163 (2C, C=N), 143-110 (18C, Ar-C), 48 (1C, -OCH_3), 29 (1C, N-CH_3). Mass (LC-MS): *m/z* 476 (M), 477 (M + 1, 100%), 478 (M + 2, 30%).

4-Thiophen-2-ylmethylene-2-(4-methoxy)phenyl-1-(5-nitro-*N*-methyl-indolin-2-one)-1*H*-imidazol-5(4*H*)-one (5d): Yield 73%, m.p.: 215-217 ºC; *m.w.*: 487.4 g/mol, m.f.: $C_{24}H_{17}N_5O_5S$, R_f: 0.69. IR (KBr, ν_{max}, cm^{-1}): 3081 (C-H *str.*, Ar), 2981, 2853, 2735 (C–H *str.*, aliph.), 2335 (C-S-C *str.*, thiophene), 1785 (C=O *str.*, indole), 17029 (C=O *str.*, imidazole), 1627 (C=N, *str.*) 1537 (C=CH *str.*), 1435 (C=C *str.*, Ar). ^{1}H NMR (DMSO) δ ppm: 8.80 (s, 1H, =CH-Ar), 8.357 (s, 1H, Ar-H), 8.064-8.048 (d, 2H, Ar-H), 7.947-7.921 (d, 2H, Ar-H), 7.820-7.757 (t, 1H, Ar-H), 7.172-7.140 (d, 2H, Ar-H), 6.889-6.869 (d, 2H, Ar-H), 3.752 (s, 3H, -OCH_3), 3.130 (s, 3H, N-CH_3). ^{13}C NMR (DMSO) δ ppm: 183, 175 (2C, CO), 167, 163 (2C, C=N), 155-124 (18C, Ar-C), 52 (1C, -OCH_3), 28 (1C, N-CH_3), 23 (1C, Ar-CH_3). Mass (LC-MS): *m/z* 487 (M), 488 (M + 1, 100%).

4-Thiophen-2-ylmethylene-2-(4-methoxy)phenyl-1-(5-fluoro-*N*-methyl-indolin-2-one)-1*H*-imidazol-5(4*H*)-one (5e): Yield 75%; m.p.: 199-201 ºC; *m.w.*: 460.2 g/mol, m.f.: $C_{24}H_{17}N_4O_3SF$, R_f: 0.69. IR (KBr, ν_{max}, cm^{-1}): 3063 (C-H *str.*, Ar), 2931, 2889, 2731 (C-H *str.*, aliph.), 2344 (C-S-C *str.*, thiophene), 1731 (C=O *str.*, indole), 1687 (C=O *str.*, imidazole), 1627 (C=N, *str.*), 1509 (C=CH *str.*), 1436 (C=C *str.*, Ar). ^{1}H NMR (DMSO) δ ppm: 8.854 (s, 1H, =CH-Ar), 7.976-7.705 (d, 2H, Ar-H), 7.6327.602 (d, 2H, Ar-H), 7.512-7.500 (d, 2H, Ar-H), 7.413-7.401 (t, 2H, Ar-H), 7.287-7.212 (d, 2H, Ar-H), 7.192 (t, 1H, Ar-H), 3.834 (s, 3H, -OCH_3), 3.234 (s, 3H, N-CH_3).

^{13}C NMR (DMSO) δ ppm: 180, 175 (2C, CO), 163, 160 (2C, C=N), 153-128 (18C, Ar-C), 55 (1C, -OCH_3), 29 (1C, N-CH_3), 24 (1C, Ar-CH_3). Mass (LC-MS): *m/z* 460 (M), 461 (M + 1, 100%), 462 (M + 2, 30%).

4-Thiophen-2-ylmethylene-2-(4-methoxy)phenyl-1-(1-ethyl-indolin-2-one)-1*H*-imidazol-5(4*H*)-one (5f): Yield: 68%, m.p.: 221-223 ºC; *m.w.*: 456.1 g/mol, m.f.: $C_{25}H_{20}N_4O_3S$, R_f: 0.58. IR (KBr, ν_{max}, cm^{-1}): 3060 (C-H *str.*, Ar), 2934, 282, 2719 (C-H *str.*, aliph.), 2365 (C-S-C *str.*, thiophene), 1766 (C=O *str.*, indole), 1709 (C=O *str.*, imidazole), 1605 (C=N, *str.*) 1515 (C=CH *str.*), 1446 (C=C *str.*, Ar). ^{1}H NMR (DMSO) δ ppm: 8.793 (s, 1H, =CH-Ar), 7.977-7.876 (d, 2H, Ar-H), 7.848-7.781 (d, 2H, Ar-H), 7.697-7.687 (d, 2H, Ar-H), 7.608-7.516 (t, 1H, Ar-H), 7.496-7.489 (d, 2H, Ar-H), 7.149 (s, 1H, Ar-H), 3.633 (s, 3H, -OCH_3), 2.913 (q, 2H, N-CH_2), 1.908 (t, 3H, N-CH_2-CH_3). ^{13}C NMR (DMSO) δ ppm: 180, 173 (2C, CO); 167, 161 (2C, C=N), 151-125 (18C, Ar-C), 51 (1C, -OCH_3), 32 (1C, N-CH_2), 18 (1C, N-CH_2-CH_3). Mass (LC-MS): *m/z* 456 (M), 457 (M + 1, 100%).

Thiophen-2-ylmethylene-2-(4-methoxy)phenyl-1-(5-chloro-*N*-ethyl-indolin-2-one)-1*H*-imidazol-5(4*H*)-one (5g): Yield 72%, m.p.: >290 ºC; *m.w.*: 490.3 g/mol, m.f.: $C_{25}H_{19}N_4O_3SCl$, R_f: 0.64, IR (KBr, ν_{max}, cm^{-1}): 3100 (C-H *str.*, Ar), 2986, 2803, 2733 (C-H *str.*, aliph.), 2382 (C-S-C *str.*, thiophene), 1762 (C=O *str.*, indole), 1714 (C=O *str.*, imidazole), 1612 (C=N, *str.*), 1523 (C=CH *str.*), 1454 (C=C *str.*, Ar), 789 (C-Cl, *str.*). ^{1}H NMR (DMSO) δ ppm: 8.786 (s, 1H, =CH-Ar), 8.102-8.002 (d, 2H, Ar-H), 7.980-7.902 (d, 2H, Ar-H), 7.789-7.658 (d, 2H, Ar-H), 7.504 (s, 1H, Ar-H), 7.238-7.201 (d, 2H, Ar-H), 7.032 (s, 1H, Ar-H), 3.654 (s, 3H, -OCH_3), 2.783 (q, 2H, N-CH_2), 2.021 (t, 3H, N-CH_2-CH_3). ^{13}C NMR (DMSO) δ ppm: 179, 173 (2C, CO); 164, 163 (2C, C=N), 156-123 (18C, Ar-C), 58 (1C, -OCH_3), 36 (1C, N-CH_2), 27 (1C, N-CH_2-CH_3), 21 (1C, -CH_3). Mass (LC-MS): *m/z* 490 (M), 491 (M + 1, 100%), 492 (M + 2, 30%).

4-Thiophen-2-ylmethylene-2-(4-methoxy)phenyl-1-(5-nitro-*N*-ethyl-indolin-2-one)-1*H*-imidazol-5(4*H*)-one (5h): Yield: 80%; m.p.: 203-205 ºC; *m.w.*: 501.4 g/mol, m.f.: $C_{25}H_{19}N_5O_5S$, R_f: 0.64, IR (KBr, ν_{max}, cm^{-1}): 3010 (C-H *str.*, Ar), 2967, 2890, 2743 (C-H *str.*, aliph.), 2344 (C-S-C *str.*, thiophene), 1760 (C=O *str.*, indole), 1710 (C=O *str.*, imidazole), 1632 (NO_2, *str.*), 1598 (C=N, *str.*) 1503 (C=CH *str.*), 1434 (C=C *str.*, Ar). ^{1}H NMR (DMSO) δ ppm: 8.668 (s, 1H, =CH-Ar), 8.004-7.980 (d, 2H, Ar-H), 7.687-7.602 (d, 2H, Ar-H), 7.590-7.502 (d, 2H, Ar-H), 7.435 (s, 1H, Ar-H), 7.230-7.224 (d, 2H, Ar-H), 7.167 (s, 1H, Ar-H), 3.489 (s, 3H, -OCH_3), 2.862 (q, 2H, N-CH_2), 1.889 (t, 3H, N-CH_2-CH_3). ^{13}C NMR (DMSO) δ ppm: 186, 176 (2C, CO); 164, 163 (2C, C=N), 153-125 (18C, Ar-C), 58 (1C, -OCH_3), 35 (1C, N-CH_2), 20 (1C, N-CH_2-CH_3). Mass (LC-MS): *m/z* 501 (M), 502 (M + 1, 100%).

4-Thiophen-2-ylmethylene-2-(4-methoxy)phenyl-1-(5-fluoro-*N*-ethyl-indolin-2-one)-1*H*-imidazol-5(4*H*)-one (5i): Yield: 69%; m.p.: 257-259 ºC; *m.w.*: 474.2 g/mol, m.f.: $C_{25}H_{19}N_4O_3SF$, R_f: 0.81. IR (KBr, ν_{max}, cm^{-1}): 3052 (C-H *str.*, Ar), 2985, 2893, 2745 (C-H *str.*, aliph.), 2345 (C-S-C *str.*, thiophene), 1762 (C=O *str.*, indole), 1704 (C=O *str.*, imidazole), 1610 (C=N, *str.*) 1520 (C=CH *str.*), 1454 (C=C *str.*, Ar), 1454 (C=C *str.*, Ar). ^{1}H NMR (DMSO) δ ppm: 8.820 (s, 1H, =CH-Ar), 8.002-7.990 (d, 2H, Ar-H), 7.748-7.702 (d, 2H, Ar-H), 7.589-7.521 (d, 2H, Ar-H), 7.489 (s, 1H, Ar-H), 7.382-7.302 (d, 2H, Ar-H), 7.232 (s, 1H, Ar-H), 3.589 (s, 3H, -OCH_3), 2.821 (q, 2H, N-CH_2), 1.890 (t, 3H, N-CH_2-CH_3). ^{13}C NMR (DMSO) δ ppm: 178, 171 (2C, CO); 166, 162 (2C, C=N), 149-123 (18C, Ar-C), 55 (1C, -OCH_3), 33 (1C, N-CH_2), 20 (1C, N-CH_2-CH_3). Mass (LC-MS): *m/z* 474 (M), 475 (M + 1, 100%), 476 (M + 2, 30%).

4-Thiophen-2-ylmethylene-2-(4-methoxy)-phenyl-1-(5-methyl-*N*-ethyl-indolin-2-one)-1*H*-imidazol-5(4*H*)-one (5j): Yield: 75%; m.p.: 219-221 ºC; *m.w.*: 470.6 g/mol, m.f.: $C_{26}H_{22}N_4O_3S$, R_f: 0.62. IR (KBr, ν_{max}, cm^{-1}): 3002 (C-H *str.*, Ar), 2968, 2883, 2754 (C-H *str.*, aliph.), 2352 (C-S-C *str.*, thiophene), 1754 (C=O *str.*, indole), 1700 (C=O *str.*, imidazole), 1612 (C=N, *str.*) 1523 (C=CH *str.*), 1438 (C=C *str.*, Ar). ^{1}H NMR (DMSO) δ ppm: ^{1}H NMR (DMSO) δ ppm: 8.650 (s, 1H, =CH-Ar), 7.901-7.900 (d, 2H, Ar-H), 7.823-7.754 (d, 2H, Ar-H), 7.565-7.501 (d, 2H, Ar-H), 7.476 (s, 1H, Ar-H), 7.324-7.302 (d, 2H, Ar-H), 7.102 (s, 1H, Ar-H), 3.602 (s, 3H, -OCH_3), 2.896 (q, 2H, N-CH_2), 2.032 (s, 3H, Ar-CH_3), 1.993 (t, 3H, N-CH_2-CH_3). ^{13}C NMR (DMSO) δ ppm: 183, 176 (2C, CO); 167, 160 (2C, C=N), 153-123 (18C, Ar-C), 53 (1C, -OCH_3), 34 (1C, N-CH_2), 21 (1C, N-CH_2-CH_3), 19 (1C, -CH_3). Mass (LC-MS): *m/z* 470 (M), 471 (M + 1, 100%).

Anticancer activity: The cell viability used to be assessing by the MTT assay with three independent experiments with six concentrations of compounds in triplicates. Cells had been trypsinized and carry out the trypan blue assay to understand potential cells in cell suspension [20]. Cells were counted by means of hemocytometer and seeded at a density of 5.0×10^3 cells/properly in 100 µL media in 96 properly plate culture medium and incubated in a single day at 37 ºC. After incubation, take off the historic media and add sparkling media 100 µL with exceptional concentrations of the test compound in labelled wells in 96 plates. After 48 h, discard the drug solution and add the fresh media with MTT solution (0.5 mg/mL^{-1}) was once added to every nicely and plates had been incubated at 37 ºC for 3 h. The optical density of solubilized crystals in DMSO used to be measured at 570 nm on a microplate reader.

The percentage growth inhibition was calculated using the following formula and the concentration of test drug wanted to inhibit cell growth by 50 % values is generated from the dose response curves for every cell line using the origin software [23,24].

$$\text{Inhibition } (\%) = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION

The target novel imidazole-5-one derivatives (**5a-j**) were synthesized in five steps by Erlenmeyer and Schiff's base mechanism. Preliminary characterization of the novel imidazole derivatives was performed by IR spectroscopy. In all compounds, the aromatic C-H stretching frequency, as expected is observed at around 3070-3000 cm^{-1} and 2960-2800 cm^{-1} for C-H aliphatic stretching frequency correspondingly. Compounds containing C-S-C moiety show strong absorption in the region of 2380-2310 cm^{-1}. The strong absorption peak observed at around 1720-1700 cm^{-1} is found to be presence of C=O stretching

frequency. The strong absorption at 1632-1610 cm^{-1} indicates the formation of C=N linkage present in the imidazole ring. In most of the compounds, the C-C stretching of the aromatic ring is around 1549-1510 cm^{-1}, respectively. The C-Cl stretching is attributed to the strong absorption in the region 810-780 cm^{-1}. The compounds containing -OCH_3 group shows peaks due to asymmetric and symmetric bending of –OCH_3 group is observed at 1260 cm^{-1} and 1053 cm^{-1}, respectively.

Similarly, ^{1}H NMR (DMSO-d_6) spectra of novel imidazole derivatives displays a singlet at δ 3.063-3.163 for *N*-methyl protons, a quartet at δ 2.783-2.913 ppm and triplet at δ 1.908-2.021 ppm for *N*-ethyl group, a singlet at δ 2.018 ppm for methylene group (Ar-CH_3). All the aromatic protons were found between δ 7.115-8.107 ppm as singlet, doublet and triplet protons. A singlet at δ 8.662-8.893 ppm for benzyl proton (Ar=CH). The carbon atoms of compounds **5a-j**, which are most affected by substitution. The chemical shifts for the carbonyl carbon (indole, imidazole) from δ 172-185 ppm and imine carbon (C=N) from δ 157-167 ppm. All aromatic carbon was found between δ 106-153 ppm of the synthesized compounds. The chemical shifts for the methoxide (Ar-OCH_3), *N*-methyl and *N*-ethyl carbon varies at δ 50-54 and δ 27-28 ppm, respectively.

Anticancer activity: The synthesized novel imidazole derivatives (**5a-j**) were screened for anticancer activity and also evaluated for cytotoxicity in conflict human breast cancer cells (MCF7) the use of MTT assay method, with doxorubicin as standard. The results suggested that each MCF7 cell lines were permit to evaluate compounds and the values are shown in Table-1. The IC_{50} values in the range of 25.33 to 162.08 µM against MCF7 cell line. Compounds **5e**, **5f** and **5j** confirmed good activity against the cell lines and are shown in Fig. 1, whereas endure all other compounds showed average activity against couple cell lines. Compounds **5e**, **5f** and **5j** possess methyl and ethyl substitution, respectively at 1st and 5th positions. Electron releasing nature of these atoms may be the possible reason for good anticancer activity [21].

TABLE-1
CYTOTOXIC ACTIVITY OF NOVEL IMIDAZOLE-5-ONE DERIVATIVES ON MCF-7 CELL

Sample Code	R	R_1	Test parameters IC_{50} [a] (µg)
			MCF-7
5a	H	-CH_3	162.08 ± 0.097
5b	-CH_3	-CH_3	76.90 ± 0.152
5c	-Cl	-CH_3	126.87 ± 0.134
5d	-NO_2	-CH_3	98.63 ± 0.163
5e	-F	-CH_3	48.19 ± 0.043
5f	-H	-C_2H_5	32.02 ± 0.165
5g	-Cl	-C_2H_5	79.84 ± 0.721
5h	-NO_2	-C_2H_5	76.24 ± 0.521
5i	-F	-C_2H_5	64.32 ± 0.032
5j	-CH_3	-C_2H_5	25.33 ± 0.172
Doxorubicin	–	–	15.52 ± 0.174

[a]All the results shown in the table are expressed as mean ± SEM of five concentrations.

Conclusion

The novel imidazole derivatives were synthesized containing indole moieties followed by Erlenmeyer and Schiff base mechanism through conventional method. The yield of the synthesized compounds was found to be in the range from 66-80%. In conclusion, the current study apotheosis the importance of imidazole derivatives having various heterocyclic moiety features superintend for anticancer activities and may additionally serve as a lead molecule for supplementary modification to obtain clinically useful novel entities.

ACKNOWLEDGEMENTS

The authors are grateful to The Head, Department of Chemistry, Talla Padmavathi College of Pharmacy and Kakatiya University, Warangal, India for providing the laboratory facilities.

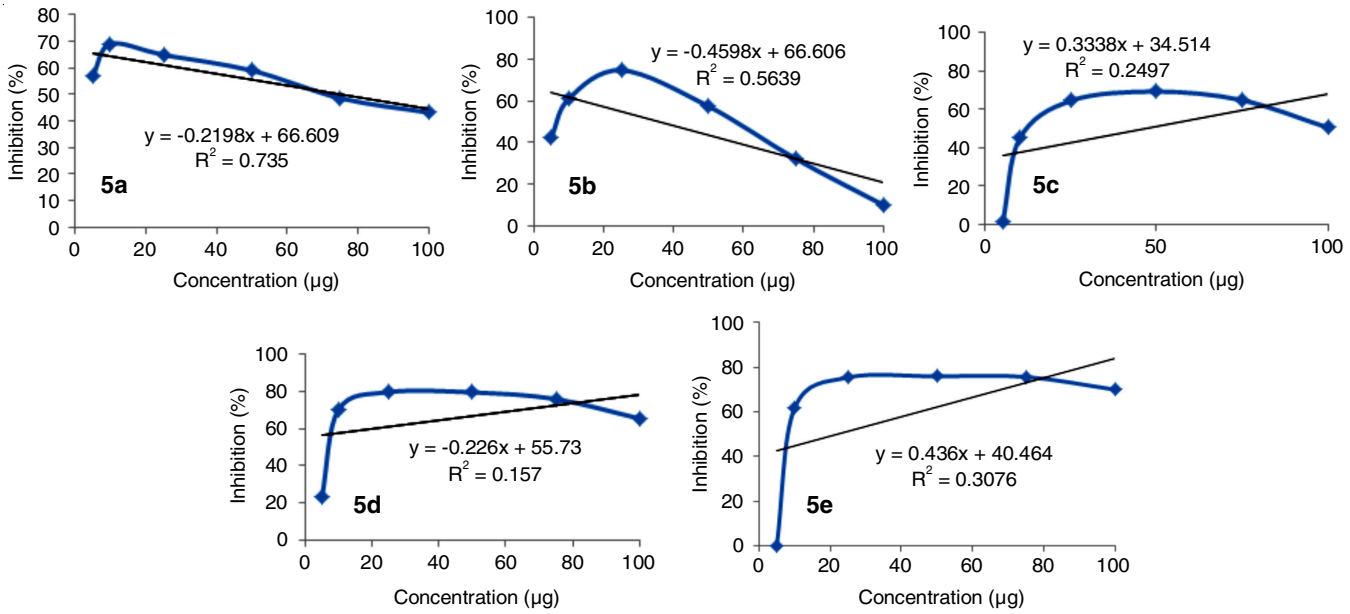


Fig. 1. Cytotoxic activity novel imidazole-5-one derivatives on MCF-7 cell

CONFLICT OF INTEREST

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

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