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Microwave-Assisted Synthesis, Molecular Docking Studies and Biological Evaluation of Benzothiazole Containing Novel Indole Derivatives

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The synthesis of novel indole derivatives **4a-o** using a microwave assisted method *via* Schiff's base and Mannich base reaction mechanism was described. Compounds **3a-c** were synthesized *via* reaction of 2-amino benzothiazole with substituted isatin by Schiff base reaction mechanism. Also, indole derivatives **4a-o** were synthesized *via* reaction of compounds **3a-c** with substituted benzaldehydes by Mannich base reaction. The biological potentials of the newly synthesized indole derivatives were evaluated for their anthelmintic activity and *in vitro* anticancer activity by MTT assay. The anticancer activity results suggested that indole derivatives **4c-o** have activity against MCF-7 and SKOV3 cells in comparison with doxorubicin as standard drug. Furthermore, the molecular docking studies of these novel derivatives of indole showed good agreement with the biological results when their binding pattern and affinity towards the active site of EGFR was also investigated.

Keywords: Isatin, 2-Amino benzothiazole, Indole derivatives, Anthelmintic activity, Anticancer activity, Mannich base.

INTRODUCTION

Indole derivatives are one of the most fortunate hetero aryl component that yielded many victorious drugs [1]. Indole moiety as well as its derivatives possessed a wide heterogeneity of pharmacological activities. Literature revealed that indole derivatives possessed antimicrobial, antiviral, antineoplastic, antihypertensive and vasodilating activities [2-4]. Furthermore, it was reported that many admixtures containing indole moiety possessed significant analgesic as well as anticancer activity [5-8]. Indole moiety fulfilled the minimum structural requirements that are needed for anticancer compounds.

In recent years, the microwave assisted organic synthesis reaction condition is promising another to conventional methods as these reactions represent clean, effective, safe, economical and eco-friendly procedure and believed to be a step towards green chemistry [9]. 2-Amino benzothioazole and indole are the class of heterocyclic compounds that has calibred special attention because it belongs to a group of substances with activity in medicinal chemistry. This bicyclic nucleus derivative are correlated with anti-inflammatory [10], anticancer [11], antimicrobial [12], antitubercular [13], antiparasitic [14], antimalarial [15], antiviral [16] and fungicidal activities [17].

So, according to this surveillance and our interest in the heterocyclic synthesis of novel active compounds against some carcinoma cell lines [18], we aimed to synthesize novel derivatives of indole as promising antitumor agents towards breast cell lines and human ovary cancer cell line (MCF7 and SKOV3) using the MTT colorimetric test [19,20] build upon their molecular docking studies *via* Schiff and Mannich bases reaction mechanism of substituted benzaldehyde and 2-aminothiazole and substituted isatin derivatives. The molecular docking study has been carried out in order to locate the viable protein ligand interactions of the dataset ligands and every docked complicated was once generated *via* Glide XP module [21,22].

EXPERIMENTAL

Fourier transform IR spectrometer (Shimadzu 8700) in the range of 4000-400 cm^{-1} using KBr pellets were used for the generation of IR spectra. ^1H NMR spectra were recorded on DPX-200 MHz NMR spectrometer using internal reference

tetramethylsilane (TMS). Mass spectra were catalogued on mass spectrophotometer (Shimadzu) by LC-MS. Precoated silica gel G plates were used to observe the progress of reaction as well as to assessment the purity of the compounds: *n*-hexane:ethyl acetate (7:3) [23]. The synthesized indole derivatives were screened for the anthelmintic and anticancer activities. For docking studies, 2D buildings of the compounds have been transformed to 3D the use of manageable algorithms and software of excessive environment friendly pressure fields. Initial geometrical optimization and strength minimization of molecules have been carried out through the usage of the Ligprep device of Schrodinger suite. Various ionization states have been generated the usage of Ligprep module the use of exceptional software EPIK alongside with quite a number feasible conformers and tautomers.

Synthesis of substituted isatin (step-1): Chloral hydrate (9 g) was added to 120 mL of water in a single necked round bottom flask. Further, added sodium sulphate (13 g) and a solution of aniline (5.4 g) in 30 mL of water containing conc. HCl (4.34 mL) to dissolve the amine. Then, added a solution of hydroxylamine hydrochloride (11 g) in 50 mL of water. Flask was then subjected for microwave irradiation for 3-4 min at 110 W. Reaction mass was allowed to stand till room temperature attained until solid mass completely converted by monitoring *via* TLC. After it, the solution containing beaker was cooled, the final product was filtered with suction pump and air dried.

Conc. sulphuric acid (10 mL, 18.4 g) was heated at 50 ºC in 250 mL conical flask and add dry isonitrosoacetolidine (2.5 g) with maintain the temperature between 60-70 ºC. External cooling was useful at this stage so that the reaction could be carried out more expeditiously after the addition of isonitroso compound was finished. Then the reaction mixture was heated to 80 ºC and maintained for about 10 min to complete the reaction. Further, the resulting the reaction mixture was cooled to room temperature and poured it into ten times its volume of cracked ice. After standing for 90 min, the final product was filtered with suction pump followed by washing with cold water to remove sulphuric acid and dried.

General procedure for synthesis of 2-aminobenzothiazole (step-2): To an equimolar concentration solution of aniline (4.6 g, 0.05 mol) and ammonium thiocyanate (3.8 g, 0.05 mol) were dissolved in 30 mL of absolute ethanol containing 4 mL of conc. HCl. Then, to this mixture, added bromine in glacial acetic acid (6.75 mL, 0.125 mol). Further, the reaction mixture was subjected on microwave irradiation for 4 min at 160 W. It was then cooled in ice bath, the obtained precipitate was filtered, washed with cold water and dried. Finally, the crude product was recrystallized from ethanol.

General procedure for synthesis of benzothiazole substituted indole derivatives (3a-c) (step-3): An equimolar (0.01 mol) mixture of 2-aminobenzothiazole, substituted isatin, catalytic amount of glacial acetic acid (15 mL) and 30 mL of ethanol was taken in 250 mL conical flask. Further, the reaction mixture was subjected to microwave irradiation for 3-4 min at 160 W. Then, the resulting reaction mixture was cooled for overnight and the precipitate was collected by filtration. Further, it was dried and recrystallized by using ethanol.

General procedure for synthesis of novel benzothiazole containing indole derivatives (4a-o) (step-4): A series of novel benzothiazole clubbed indole derivatives was synthesized by the reaction of equimolar (0.01 mol) mixture of benzothiazole substituted indole derivatives (**3a-c**) and substituted benzaldehyde, formaldehyde and acetanilide and 30 mL of DMF in 250 mL conical flask. Further, the reaction mixture was put on view for microwave irradiation for 10 cycles for 10 s (3 min) at 180 W. The resulting reaction mixture was cooled, left to overnight and then precipitated solid was obtained by filtration, dried and recrystallized from ethanol (**Scheme-I**).

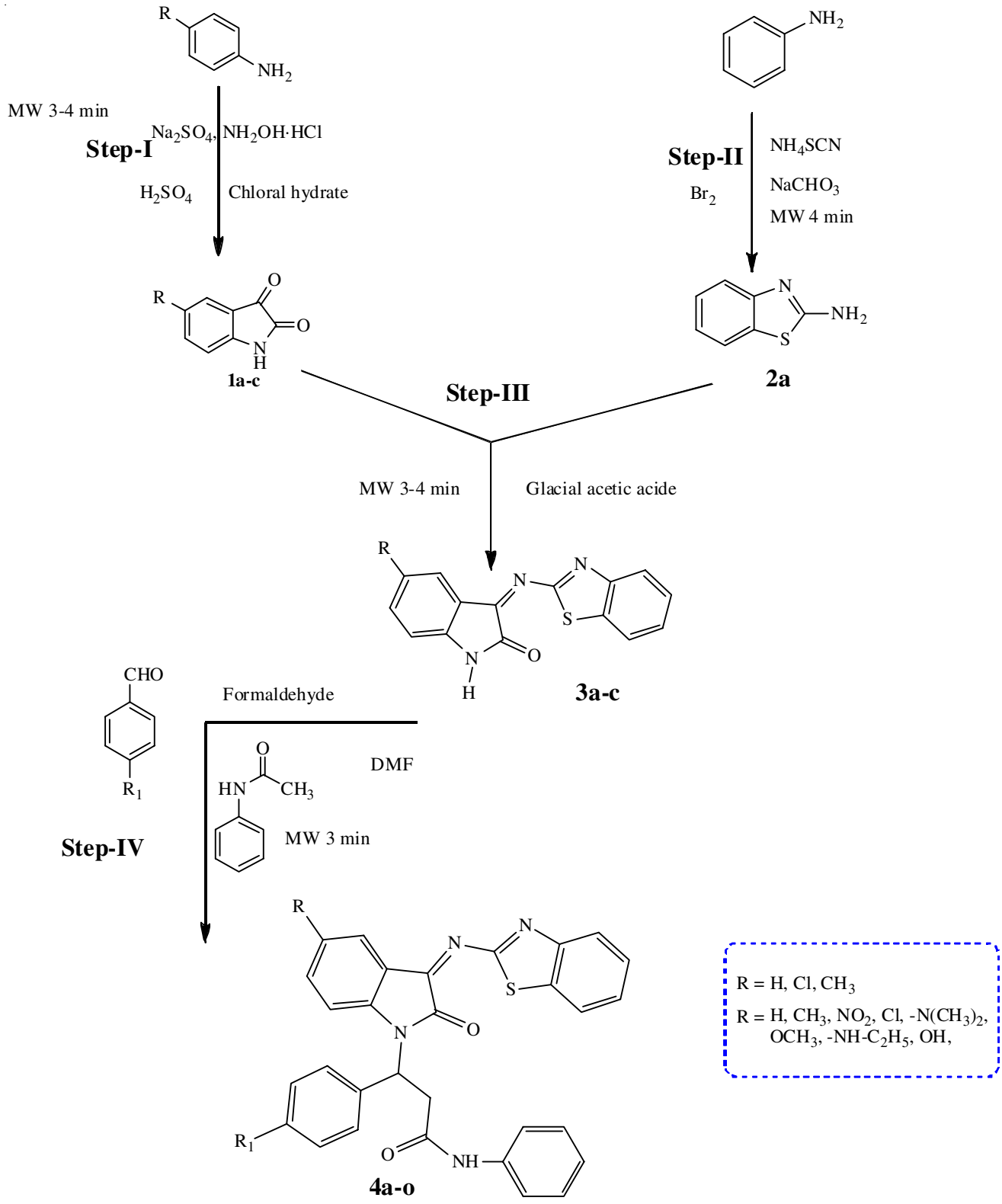
3-(3-(Benzo[*d*]thiazol-2-ylimino)-2-oxoindolin-1-yl)-*N*-phenyl-3-phenyl propanamide (4a): m.p.: 219-221 ºC; m.f.: $C_{30}H_{22}N_4O_2S$, yield 76%. IR (KBr, ν_{max}, cm^{-1}): 3409-NH *str.*, acetamide), 3009 (-CH *str.*, aromatic), 2958 (-CH *str.*, propanamide), 2767 (-CH_2 *str.*, alkyl), 1737 (C=O *str.*), 1511 (C=N *str.*), 1446 (C=C *str.*, Ar), 1327 (C-N *str.*). 11.73 (1H, -NH amide), 8.47-6.86 (18H, Ar-H), 4.36 (2H, -CH_2, propanamide), 2.07 (1H, -CH_2 in propanamide). Mass (LC-MS): *m/z* 502 (M), 503 (M + 1, 100%).

3-(3-(Benzo[*d*]thiazol-2-ylimino)-2-oxoindolin-1-yl)-*N*-phenyl-3-(*p*-tolyl)propanamide (4b): m.p.: 203-205 ºC; m.f.: $C_{31}H_{24}N_4O_2S$, yield 70%, IR (KBr, ν_{max}, cm^{-1}): 3427-NH *str.*, propanamide), 3089 (-CH *str.*, aromatic), 2986 (-CH *str.*, propanamide), 2881, 2728 (-CH_2 *str.*, alkyl), 1719 (C=O *str.*), 1570 (C=N *str.*), 1328 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 11.01 (1H, -NH amide), 8.49-7.14 (17H, Ar-H), 4.50 (2H, -CH_2 in propanamide), 2.30 (3H, -CH_3), 2.15-2.10 (1H, -CH- propanamide). Mass (LC-MS): *m/z* 516 (M), 517 (M + 1, 100%).

3-(3-(Benzo[*d*]thiazol-2-ylimino)-2-oxoindolin-1-yl)-*N*-phenyl-3-(*p*-methoxy)phenyl propanamide (4c): m.p.: 231-233 °C; m.f.: $C_{33}H_{24}N_4O_3S$, yield 84%, IR (KBr, ν_{max}, cm^{-1}): 3382 (-NH *str.*, acetamide), 3082 (-CH *str.*, aromatic), 2925 (-CH *str.*, propanamide), 2825 (-CH_2 *str.*, alkyl), 1725 (C=O *str.*), 1519 (C=N *str.*), 1336 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 11.14 (1H, -NH amide), 8.37-7.51 (17H, Ar-H), 4.34-4.30 (2H, -CH_2 in propanamide), 3.37-3.33 (3H, OCH_3). 2.15-2.10 (1H, -CH- in propanamide).

3-(3-(Benzo[*d*]thiazol-2-ylimino)-2-oxoindolin-1-yl)-*N*-phenyl-3-(*p*-nitro)phenyl propanamide (4d): m.p.: 178-181 ºC; m.f.: $C_{30}H_{21}N_5O_4S$, yield 81%, IR (KBr, ν_{max}, cm^{-1}): 3401 (-NH *str.*, propanamide), 3094 (-CH *str.*, aromatic), 2917 (-CH *str.*, propanamide), 2873, 2747 (-CH_2 *str.*, alkyl), 1715 (C=O *str.*), 1584 (C=N *str.*), 1328 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 11.03 (1H, -NH amide), 8.56-7.32 (17H, Ar-H), 4.32 (2H, -CH_2 in propanamide), 2.23-2.210 (1H, -CH- propanamide). Mass (LC-MS): *m/z* 547 (M), 548 (M + 1, 100%).

3-(3-(Benzo[*d*]thiazol-2-ylimino)-2-oxoindolin-1-yl)N-phenyl-3-(*p*-chloro)phenyl propanamide (4e): m.p.: 231-233 ºC; m.f.: $C_{30}H_{21}N_4O_2S$, yield 78%, IR (KBr, ν_{max}, cm^{-1}): 3421 (-NH *str.*, acetamide), 3063 (-CH *str.*, aromatic), 2932 (-CH *str.*, propanamide). 2878 (-CH_2 *str.*, alkyl), 1721 (C=O *str.*), 1532 (C=N *str.*), 1376 (C-N *str.*), 786 (C-Cl, *str.*). ^{1}H NMR (DMSO) δ ppm: 11.32 (1H, -NH amide), 8.46-7.32 (17H, Ar-H), 4.54-4.51 (2H, -CH_2 in propanamide), 3.36-3.30 (3H, OCH_3), 2.23-2.20 (1H, -CH- in propanamide). Mass (LC-MS): *m/z* 536 (M), 537 (M + 1, 100%), 538 (M + 2, 30%).



Scheme-I

3-(3-(Benzo[*d*]thiazol-2-ylimino)-2-oxoindolin-1-yl)-*N*-phenyl-3-(*p*-dimethyl amino)phenyl propanamide (4f): m.p.: 239-241 ºC; m.f.: $C_{32}H_{27}N_5O_2S$, yield 72%. IR (KBr, ν_{max}, cm^{-1}): 3421-NH *str.*, acetamide), 3020 (-CH *str.*, aromatic), 2987 (-CH *str.*, propanamide), 2793 (-CH_2 *str.*, alkyl), 1723 (C=O *str.*), 1528 (C=N *str.*), 1453 (C=C *str.*, Ar), 1343 (C-N *str.*). 11.02 (1H, -NH amide), 8.23-8.012 (19H, Ar-H), 4.23-4.19 (2H, -CH_2, propanamide), 2.675-2.601 (6H, -N9CH_3)$_2$, 2.01 (1H, -CH_2 in propanamide). Mass (LC-MS): *m/z* 545 (M), 546 (M + 1, 100%).

3-(3-(Benzo[*d*]thiazol-2-ylimino)-2-oxoindolin-1-yl)-*N*-phenyl-3-(*p*-hydroxy)phenyl propanamide (4g): m.p.: 261-263 ºC; m.f.: $C_{30}H_{22}N_4O_3S$, yield 76%, IR (KBr, ν_{max}, cm^{-1}): 3645 (OH *str.*, Ar-OH), 3428-NH *str.*, propanamide), 3081 (-CH *str.*, aromatic), 2993 (-CH *str.*, propanamide), 2890, 2756 (-CH_2 *str.*, alkyl), 1704 (C=O *str.*), 1543 (C=N *str.*), 1347 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 11.25 (1H, -NH amide), 8.35-7.28 (17H, Ar-H), 4.32 (2H, -CH_2 in propanamide), 2.28 (3H, -CH_3), 2.13-2.10 (1H, -CH- propanamide). Mass (LC-MS): *m/z* 518 (M), 519 (M + 1, 100%).

3-(3-(Benzo[*d*]thiazol-2-ylimino)-(5-methyl)-2-oxoindolin-1-yl)-*N*-phenyl-3-phenyl- propanamide (4h): m.p.: 235-237 ºC; m.f.: $C_{31}H_{24}N_4O_2S$, yield 63%, IR (KBr, ν_{max}, cm^{-1}): 3410 (-NH *str.*, acetamide), 3073 (CH *str.*, aromatic), 2902 (-CH *str.*, propanamide), 2878 (-CH_2 *str.*, alkyl), 1717 (C=O *str.*), 1533 (C=N *str.*), 1328 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 11.46 (1H, -NH amide), 8.20-7.34 (17H, Ar-H), 4.42-4.37 (2H, -CH_2 in propanamide), 3.28-3.19 (3H, OCH_3). 2.29-2.17 (1H, -CH- in propanamide), 2.002 (3H, -CH_3). Mass (LC-MS): *m/z* 516 (M), 517 (M + 1, 100%).

3-(3-(Benzo[*d*]thiazol-2-ylimino)-(5-methyl)-2-oxoindolin-1-yl)-*N*-phenyl-3-(*p*-tolyl)propanamide (4i): m.p.: 199-201 ºC; m.f.: $C_{32}H_{26}N_4O_2S$, yield 78%, IR (KBr, ν_{max}, cm^{-1}): 3401 (-NH *str.*, propanamide), 3094 (-CH *str.*, aromatic), 2917 (-CH *str.*, propanamide), 2873, 2747 (-CH_2 *str.*, alkyl), 1715 (C=O *str.*), 1584 (C=N *str.*), 1328 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 11.03 (1H, -NH amide), 8.56-7.32 (17H, Ar-H), 4.32 (2H, -CH_2 in propanamide), 2.23-2.210 (1H, -CH- propanamide), 2.092-2.001 (6H, -CH_3). Mass (LC-MS): *m/z* 530 (M), 531 (M + 1, 100%).

3-(3-(Benzo[*d*]thiazol-2-ylimino)-(5-methyl)-2-oxoindolin-1-yl)-*N*-phenyl-3-(*p*-methoxy)phenyl propanamide (4j): m.p.: 251-253 ºC; m.f.: $C_{32}H_{26}N_4O_2S$, yield 80%, IR (KBr, ν_{max}, cm^{-1}): 3421 (-NH *str.*, acetamide), 3063 (-CH *str.*, aromatic), 2932 (-CH *str.*, propanamide). 2878 (-CH_2 *str.*, alkyl), 1721 (C=O *str.*), 1532 (C=N *str.*), 1376 (C-N *str.*), 786 (C-Cl, *str.*). ^{1}H NMR (DMSO) δ ppm: 11.32 (1H, -NH amide), 8.46-7.32 (17H, Ar-H), 4.54-4.51 (2H, -CH_2 in propanamide), 3.36-3.30 (3H, OCH_3), 2.23-2.20 (1H, -CH- in propanamide), 2.032 (3H, -CH_3). Mass (LC-MS): *m/z* 546 (M), 546 (M + 1, 100%).

3-(3-(Benzo[*d*]thiazol-2-ylimino)-(5-methyl)-2-oxoindolin-1-yl)-*N*-phenyl-3-(*p*-nitro)phenyl propanamide (4k): m.p.: 215-217 ºC; m.f.: $C_{31}H_{24}N_4O_3S$, yield 73%. IR (KBr, ν_{max}, cm^{-1}): 3412-(NH *str.*, acetamide), 3023 (-CH *str.*, aromatic), 2976 (-CH *str.*, propanamide), 2798 (-CH_2 *str.*, alkyl), 1716 (C=O *str.*), 1523 (C=N *str.*), 1453 (C=C *str.*, Ar), 1342 (C-N *str.*), 11.02 (1H, -NH amide), 8.318-7.023 (18H, Ar-H), 4.27-4.20 (2H, -CH_2, propanamide), 2.203 (3H, -CH_3), 2.14 (1H, -CH_2 in propanamide). Mass (LC-MS): *m/z* 532 (M), 533 (M + 1, 100%).

3-(3-(Benzo[*d*]thiazol-2-ylimino)-(5-methyl)-2-oxoindolin-1-yl)-*N*-phenyl-3-(*p*-hydroxy)phenyl propanamide (4l): m.p.: 223-225 ºC; m.f.: $C_{31}H_{24}N_4O_2S$, yield 67%, IR (KBr, ν_{max}, cm^{-1}): 3658 (-OH, *str.*, Ar-OH), 3443 (-NH *str.*, propanamide), 3092 (-CH *str.*, aromatic), 2974 (-CH *str.*, propanamide), 2854, 2783 (-CH_2 *str.*, alkyl), 1710 (C=O *str.*), 1573 (C=N *str.*), 1354 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 12.01 (1H, -NH amide), 8.28-7.046 (16H, Ar-H), 4.34 (2H, -CH_2 in propanamide), 2.63 (3H, -CH_3), 2.23-2.20 (1H, -CH- propanamide). Mass (LC-MS): *m/z* 516 (M), 517 (M + 1, 100%).

3-(3-(Benzo[*d*]thiazol-2-ylimino)-(5-nitro)-2-oxoindolin-1-yl)-*N*-phenyl-3-phenyl propanamide (4m): m.p.: 261-263 ºC; m.f.: $C_{30}H_{21}N_5O_4S$, yield 82%, IR (KBr, ν_{max}, cm^{-1}): 3399 (-NH *str.*, acetamide), 3102 (-CH *str.*, aromatic), 2942 (-CH *str.*, propanamide), 2883 (-CH_2 *str.*, alkyl), 1728 (C=O *str.*), 1643 (NO_2, *str.*), (1537 (C=N *str.*), 1336 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 11.35 (1H, -NH amide), 8.29-8.102 (16H, Ar-H), 4.42-4.41 (2H, -CH_2 in propanamide), 3.56-3.51 (3H, OCH_3), 2.268-2.341 (1H, -CH- in propanamide).

3-(3-(Benzo[*d*]thiazol-2-ylimino)-(5-nitro)-2-oxoindolin-1-yl)-*N*-phenyl-3-(*p*-methyl)phenyl propanamide (4n): m.p.: 255-257 ºC; m.f.: $C_{31}H_{23}N_5O_4S$, yield 77%, IR (KBr, ν_{max}, cm^{-1}): 3411 (-NH *str.*, propanamide), 3003 (-CH *str.*, aromatic), 2967 (-CH *str.*, propanamide), 2894, 2783 (-CH_2 *str.*, alkyl), 1705 (C=O *str.*), 1634 (NO_2, *str.*), (1574 (C=N *str.*), 1348 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 11.632 (1H, -NH amide), 8.253-7.042 (16H, Ar-H), 4.283-4.201 (2H, -CH_2 in propanamide), 2.329-2.302 (1H, -CH- propanamide), 2.032 (3H, -CH_3). Mass (LC-MS): *m/z* 561 (M), 562 (M + 1, 100%).

3-(3-(Benzo[*d*]thiazol-2-ylimino)-(5-nitro)-2-oxoindolin-1-yl)-*N*-phenyl-3-(*p*-methoxy)phenyl propanamide (4o): m.p.: 209-2113 ºC; m.f.: $C_{31}H_{23}N_5O_5S$, yield 74%, IR (KBr, ν_{max}, cm^{-1}): 4c: 3453 (-NH *str.*, acetamide), 3920 (-CH *str.*, aromatic), 2947 (-CH *str.*, propanamide). 2890 (-CH_2 *str.*, alkyl), 1702 (C=O *str.*), 1545 (C=N *str.*), 1387 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 11.732 (1H, -NH amide), 8.290-7.102 (16H, Ar-H), 4.483-4.721 (2H, -CH_2 in propanamide), 2.281-2.152 (3H, CH_3), 2.109-2.013 (1H, -CH- in propanamide). Mass (LC-MS): *m/z* 577 (M), 578 (M + 1, 100%).

Pharmacological activity

Anticancer activity: Cell viability was once evaluated by means of the MTT assay with three impartial experiments with six concentrations of compounds in triplicates. Cells have been trypsinized and function the trypan blue assay to be aware of doable cells in cellphone suspension. Cells had been counted by way of hemocytometer and seeded at a density of 5.0×10 three cells/properly in 100 µL media in 96-properly plate subculture medium and incubated in a single day at 37 ºC. After incubation, take off the ancient media and add clean media a 100 µL with one of kind concentrations of the take a look at compound in labeled wells in 96-plates. After 48 h, discard the drug answer and add the clean medic with MTT answer (0.5 mg mL^{-1}) was once introduced to every properly and plates have been incubated at 37 ºC for 3 h. At the give up of incubation time, precipitates are fashioned as an end result of the discount of the MTT salt to chromophore formosan crystals by way of the cells with metabolically energetic mitochondria. The optical density of solubilized crystals in DMSO used to be measured at 570 nm on a microplate reader [23].

The share increase inhibition was once calculated the use of the following system and the awareness of take a look at drug required to inhibit mobile boom through 50% values was generated from the dose-response curves for every mobile line the usage of with starting place software program [24].

Anthelmintic activity: The synthesized compounds were screened for anthelminthic recreation by using the usage of earth worms. Six earthworms of almost equal measurement had been positioned in preferred drug answer and take a look at compound's options at room temperature. Normal saline used as control. The widespread drug and check compounds have been dissolved in minimal volume of dimethyl sulfoxide (DMSO) and adjusted the extent up to 10 mL with regular saline answer to get the attention of 0.1% w/v, 0.2% w/v and 0.5% w/v [24]. Albendazole was once used as a widespread drug.

Molecular docking studies: The digital shape of the EGFR kinase area used to be retrieved from the Protein databank internet site with PDB Id: 1M17 and the shape used to be optimized by deleting unbound water molecules which are over 1 Å, including hydrogen atoms to fulfil the valences, including lacking amino acids to stabilize facet chains and electricity of the complete shape was once minimized the usage of OPLS-2005 pressure discipline the usage of Protein Preparation Wizard device of Schrodinger Suite [25].

Thus structurally optimized protein shape was once used to observe protein-ligand interactions of the dataset ligands the use of Glide Xp docking protocol. Initially, a 3D grid used to be set up to the binding pocket (active site) of the protein, into which all the dataset ligands had been docked into. Binding interactions and effectively of the binding have been calculated in phrases of Glide Score, which is a mixture of hydrophilic, hydrophobic, steel binding groups, van der Waals energy, freezing rotatable bonds and polar interactions with receptor.

GScore = 0.065 × Van der Waals strength + 0.130 × Coulomb power + Lipophilic time period (Hydrophobic interactions) + H bonding + Metal binding + BuryP (Penalty for buried polar groups) + RotB (Penalty for freezing rotatable bonds) + Site (Polar interactions in the lively site).

RESULTS AND DISCUSSION

A series of novel indole derivatives were synthesized by the Schiff and Mannich base reaction mechanisms. Various substituted isatin derivatives (**1a-c**) reacted with 2-amino benzothiazole (**2**) in the presence of glacial acetic acid to give compounds (**3a-c**). Further, these compounds undergo Mannich base reaction with substituted aniline, substituted aldehyde and acetanilide to give the final derivatives (**4a-o**). The synthesized novel compounds Mannich were screened for anticancer and anthelmintic activities. All the newly synthesized compounds (**4a-o**) were characterized based on analytical and spectral data including IR, LC-MASS and ^{1}H NMR data.

Preliminary characterization of the novel indole derivatives (**4a-o**) was performed by IR spectroscopy. Practically, in all the compounds have N-H stretching frequency observed at 3460-3400 cm^{-1}, the aromatic and aliphatic C-H stretching frequency, as expected is observed at around 3070-3000 cm^{-1} and 2960-2800 cm^{-1} correspondently. Compounds containing O-H group show strong absorption in the region of 3650-3500 cm^{-1}. The strong absorption peak observed at around 1740-1700 cm^{-1} is observed due to the presence of C=O stretching frequency and in most of the compounds the C-C stretching of the aromatic ring is around 1550-1515 cm^{-1} respectively. The C-Cl stretching is attributed to the strong absorption in the region 813-782 cm^{-1} and some compounds containing -OCH_3 group shows peaks due to asymmetric and symmetric bending of –OCH_3 group is observed at around 1265 cm^{-1} and 1058 cm^{-1}, respectively.

Similarly, the ^{1}HNMR (DMSO-d_6) spectra of novel indole derivatives showing a singlet at δ 11.00-12.00 ppm for NH protons. All the derivatives display a triplet and singlet at δ 4.200-4.630 ppm for propanamide and propane CH protons, a singlet at δ 2.00-2.210 ppm for methylene group (Ar-CH_3). All the aromatic protons were found between δ 8.434-7.002 ppm as singlet, doublet and triplet protons. The carbon atoms of compounds **4a-o**, which are most affected by substituted groups.

Anticancer activity: Novel indole derivatives were evaluated for cytotoxicity against human breast cancer cells (MCF7) and human ovary cancer cell line (SKOV3) using MTT assay, with doxorubicin as standard. Results (Table-1) proposed that both cell lines were susceptible to the evaluated compounds. Compounds **4c-o** showed IC_{50} values in the range of 8.5 µM to 23.7 µM against MCF7 cell line and 7.4 µM to 23.7 µM against SKOV3 cell line. Compounds **4c**, **4d**, **4e** and **4k** showed good activity against the cell lines, whereas, remaining all other compounds showed moderate activity against both cell lines. All the results are expressed as a mean ± SEM of five concentrations.

Anthelmintic activity: Novel indole compounds (**4a-o**) were evaluated for anthelmintic activity on Indian earthworms (*Pheretima posthuma*). All the synthesized compounds showed anthelmintic activity (Table-2), among these compounds, all compounds showed a significant paralytic time of earthworms, compared to standard drug albendazole at 0.1%, 0.2% and 0.5% concentrations of compounds. A closer inspection of data indicated that the synthesized compounds **4c**, **4g**, **4d**, **4k** and **4o** showed good anthelmintic activities whereas others showed significant activities. After all, the synthesized compounds in overall investigation confirmed the better activity against *Peritima posthuma*.

Molecular docking studies: Molecular docking studies have been carried out in order to locate the viable protein ligand interactions of the dataset ligands. Additionally, these additionally assisted in finding out the conformational adjustments of the ligand in the protein environment. About generates one hundred distinctive protein ligand complicated conformations for every docked complicated was once generated *via* Glide XP module. Based on the E Model energy, solely one was once displayed in the result. Glide dock sores of the dataset ligands have been proven in Table-3 alongside with the interplay amino acids and quantity of amino acids.

Among the docked ligands, compound **4f** pronounced perfect dock rating of -4.689 with E mannequin strength of -76.92 Kcal/mol (Fig. 1). Dock rankings of all the compounds ranged from -4.689 (compound **4f**) to -3.27 (compound **4j**). All the docked ligands have proven single H-bond between ASP 831 and amide team in the ligands, whereas, the docking rating of compound **4k** is totally based on the solely non-hydrophilic interactions. Hydrophobic interactions have been

TABLE-1
PHYSICAL DATA AND ANTIHELMINTIC ACTIVITY OF NOVEL INDOLE DERIVATIVES (**4a-o**)

Compd. No.	m.f.	R	R_1	R_f value	Time (min)					
					For paralysis % concentration			For death % concentration		
					0.1	0.2	0.5	0.1	0.2	0.5
4a	$C_{30}H_{22}N_4O_2S$	H	H	0.70	20	19	20	52	47	35
4b	$C_{31}H_{24}N_4O_2S$	H	CH_3	0.78	31	23	19	52	45	33
4c	$C_{33}H_{24}N_4O_3S$	H	OCH_3	0.59	20	14	11	47	39	30
4d	$C_{30}H_{21}N_5O_4S$	H	NO_2	0.70	23	21	18	45	38	31
4e	$C_{30}H_{21}N_4O_2S$	H	Cl	0.79	22	19	18	53	42	32
4f	$C_{32}H_{27}N_5O_2S$	H	$N(CH_3)_2$	0.68	23	16	19	52	37	31
4g	$C_{30}H_{22}N_4O_3S$	H	OH	0.60	21	24	20	48	37	32
4h	$C_{31}H_{24}N_4O_2S$	CH_3	H	0.74	18	18	16	54	42	30
4i	$C_{32}H_{26}N_4O_2S$	CH_3	CH_3	0.83	25	19	19	52	47	35
4j	$C_{32}H_{26}N_4O_3S$	CH_3	OCH_3	0.65	24	20	17	53	45	37
4k	$C_{31}H_{24}N_4O_3S$	CH_3	NO_2	0.78	20	14	11	47	39	30
4l	$C_{31}H_{24}N_4O_2S$	CH_3	OH	0.82	19	16	13	45	35	29
4m	$C_{30}H_{21}N_5O_4S$	NO_2	H	0.70	19	19	15	47	37	32
4n	$C_{31}H_{23}N_5O_4S$	NO_2	CH_3	0.78	26	23	19	53	47	36
4o	$C_{31}H_{23}N_5O_5S$	NO_2	OCH_3	0.85	20	14	11	48	35	32
Control	–	–	–	–	–	–	–	–	–	–
Albendazole	–	–	–	–	15	12	8	44	34	26

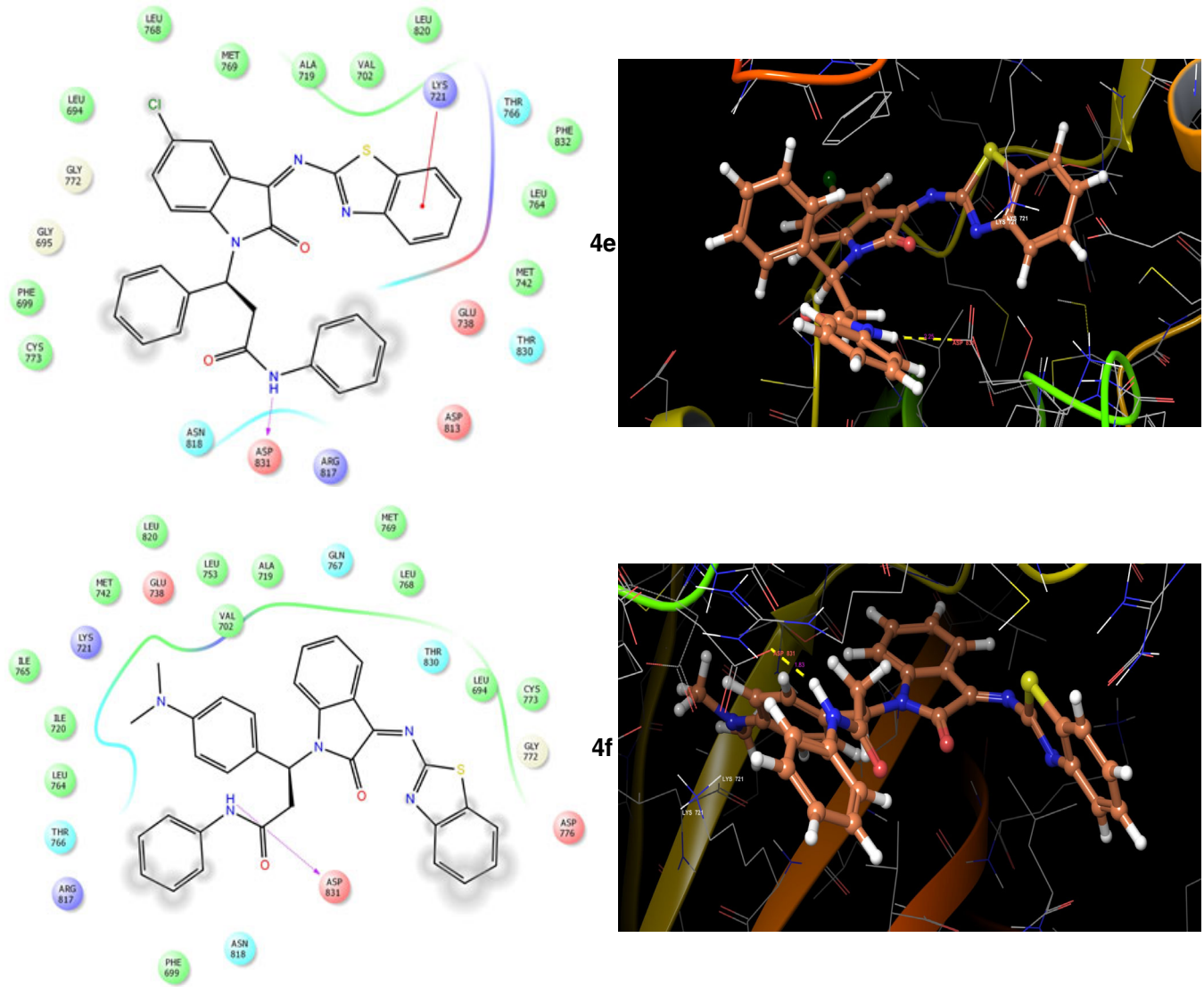


Fig. 1. Binding modes of compounds **4e** and **4f** onto the proteins

TABLE-2
CYTOTOXIC ACTIVITY OF NOVEL INDOLINE DERIVATIVES ON MCF-7 AND SKVO3 CELLS

Compd. No.	IC_{50} values (μM)	
	MCF7	SKOV3
4c	9.5 ± 0.19	10.2 ±0.02
4d	10.1 ± 0.12	14.4 ± 0.25
4e	8.5± 0.24	9.5 ± 0.21
4f	13.2 ± 0.31	10.1 ± 0.56
4e	12.3 ± 0.09	20.4 ± 0.21
4f	23.7 ± 0.21	23.7 ± 0.27
4k	7.4 ± 0.65	13.2± 0.42
4o	12.3 ± 0.54	07.4±0.53
Doxorubicin	0.8	0.7

TABLE-3
in silico EGFR INHIBITION OF NOVEL INDOLE DERIVATIVES-GLIDE DOCK SORES OF THE DATASET LIGANDS

Comp. No.	Dock score XP GScore	No of H-bonds	Interacting amino acids	H-bond lengths (Å)	Emodel energy
4f	-4.689	1	ASP 831	1.83	-76.92
4k	-4.427	0	–	–	-72.451
4e	-4.317	1	ASP 831	2.25	-79.348
4c	-3.95	1	ASP 831	1.94	-66.174
4j	-3.27	1	ASP 831	1.91	-77.984

discovered between benzothiazole businesses of compounds **4e** and **4k** with LYS 721.

Conclusion

In conclusion, novel benzothiazole containing indole derivatives substituted isatin and 2-aminobenzothiazole followed by Mannich base reaction under microwave irradiation were reported. The yield of the synthesized compounds was found to be in the range from 64-83%. The microwave method proved an easy, reduces reaction times with higher yields and all the synthesized compounds showed good anthelmintic and anti-cancer activities. Molecular docking studies were performed to determine the protein-ligand interactions and also to understand the conformational changes in the protein-ligand complex. Docking scores of these compounds were ranged between -3.27 to -4.689.

CONFLICT OF INTEREST

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

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