Original Article

N-(2-(1H-benzo[d]imidazol-2-yl)Phenyl)-2-(Substituted-styryl)Aniline as Anti-proliferative Agents: Rejuvenating the Importance of Low Molecular Weight Ligands in Oncotherapeutics

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ABSTRACT

Background: The rationale behind the study involved that in individuality benzimidazole-based molecules demonstrates significant anti-proliferative activity; chalcone molecules like xanthohumol are known to express noteworthy anti-cancer activity; benzamide derived products show remarkable inhibition of HDAC (an emerging anti-proliferative target) and styrene-based compounds possesses notable anti-tumor activity. **Materials and Methods:** In this research, an attempt was made to synthesize and characterize a series of hybridized molecules of the prototype *(E)-N*-(2-(1*H*-benzo[d]imidazol-2-yl) phenyl)-2-(substituted-styryl)aniline which comprises of a benzimidazole function; along with a chalcone (or styryl) moiety linked by a benzamide. The study involved screening of the novel derivatives against non-small cell lung cancer cell line (H460; ATCC: HTB177) and human colorectal cancer cell line (HCT116; ATCC: CCL-247) using Propidium Iodide assay. *In silico* docking study was also performed against protein tyrosine kinase (PDB ID: 2J5F) to determine the probable mechanism of action of the novel compounds. **Results:** The study reflected the profound role and positions of substitution on the phenyl moiety of the benzimidazole system. The compound DSTYR4 displayed most potent anti-proliferative activity with IC_{50} values of 2.98 μM against HCT116 cell line and 5.15 μM against H460 cell line. **Conclusion:** The research fruitfully rejuvenates the potentials and importance of small molecular weight ligands for experimental oncology.

Key words: Benzimidazole, Benzamide, Styryl, Chalcone, Hybrid, Anti-proliferative.

INTRODUCTION

Cancer is the leading cause of mortality and morbidity after the heart diseases across the globe which affected 8.2 million lives in the year 2012.[1] Despite enormous efforts in developing newer leads and novel chemotherapeutic strategies for treating various forms of cancer, this disease remained the key concern across the globe. The need for an urgent alternative or a search for unexplored classes of substances against cancer cells remained the foremost need amongst scientists, of which regulation of cellular proliferation is the important approach to understanding and regulate cancer.

Low Molecular Weight Ligands (LMWL) have gained popularity in the modulation of several targets owing to their therapeutic smart characteristics. Heterocyclics, bi-cyclic scaffolds, or hybridized compounds formed by the fusion of a heterocyclic scaffold with another molecule have shown better therapeutic activity than their parents, leading to their popularity in modern chemotherapeutics. Benzimidazole scaffold has offered

Submission Date: 12-07-2019;
Revision Date: 31-12-2019;
Accepted Date: 30-01-2020

DOI: 10.5530/ijper.54.2.49

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several biological activities like anticancer,[2] anti-microbial,[3] anti-inflammatory,[4] antimalarial,[5] anti-hypertensive,[6] etc. Several marketed drugs such as albendazole, mebendazole, tiabendazole, fenbendazole, lansoprazole, omeprazole, atacand, telmisartan, candesartan, mizolastine, bilastine, etc. are the classic examples of 1,2- or 1,3-disubstituted benzimidazole.[7] Benzimidazole remained one of the most promising anti-cancer heterocyclic scaffolds which demonstrated successful anti-proliferative activity along with multifaceted attributes like inhibition of kinases,[8] interaction with nuclear targets,[9] increase sensitivity to chemotherapy,[10] reduces the chances of metastasis[11] and reduces resistance of drug influx.[12] A number of benzimidazole hybrids like oxindole,[13] quinazoline,[14] coumarin,[15] pyrazole,[16] etc. have demonstrated excellent activity and have reached the hall of fame at this moment.

Naturally occurring compounds having styryl function have started attracting the medicinal chemists in synthesizing diverse styryl compounds and sequentially screen them for various pharmacological activity. One of such attempt gave 1-substituted-4-styryl[1,2,4] triazolo-[4,3-a]quinoxaline derivatives,[17] when these compounds were tested for their biological importance, found to possess anticonvulsant activity. Once synthetic compounds have proven the pharmacological importance, large numbers of styryl substituted compounds were synthesized with the hope to obtain biologically important molecules. A large number of compounds are known to exist having styryl function attached to different heterocyclic nucleuses. From the structures of naturally obtained styryl compounds and their importance as anticancer agents, medicinal chemists then started to synthesize compounds containing styryl function attached to different heterocyclic rings and screened them for cytotoxic potential.

The rationale behind the study involved that in individuality benzimidazole-based molecules demonstrates significant anti-proliferative activity; chalcone molecules like xanthohumol are known to express noteworthy anti-cancer activity; benzamide derived products show remarkable inhibition of HDAC (an emerging anti-proliferative target) and styrene-based compounds possesses notable anti-tumor activity (Figure 1).[2,18-20] In this research, an attempt was made to synthesize and characterize a series of hybridized molecules of the prototype *(E)-N*-(2-(1*H*-benzo[d]imidazol-2-yl) phenyl)-2-(substituted-styryl)aniline which comprises of a benzimidazole function; along with a chalcone (or styryl) moiety linked by a benzamide. The study involved screening of the novel derivatives against non-small cell lung cancer cell line (H460; ATCC: HTB177) and

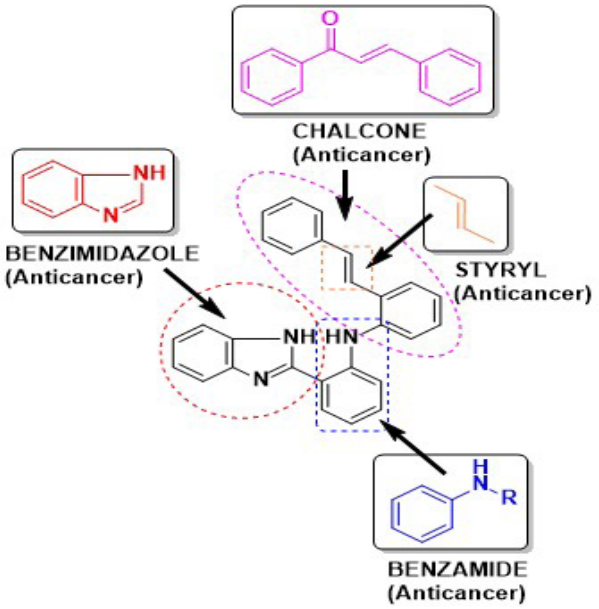


Figure 1: The rationale behind the designing of *(E)-N*-(2-(1H-benzo[d]imidazol-2-yl)phenyl)-2-(substituted-styryl)aniline derivatives.

human colorectal cancer cell line (HCT116; ATCC: CCL-247) using Propidium Iodide assay. *In silico* docking study was also performed against protein tyrosine kinase (PDB ID: 2J5F) to determine the probable mechanism of action of the novel compounds.

MATERIALS AND METHODS

Instrumentation

All chemicals used for synthesis were purchased from Sigma-Aldrich and Merck. All other solvents and reagents were of analytical grade were procured from various commercial sources. Melting points were measured on Perfit melting point apparatus and are uncorrected. The infrared spectra were recorded in KBr discs on Win IR FTS 135 instrument. The ^{1}H-NMR (400 MHz) spectra were recorded by Bruker spectrospin DPX-300 NMR using TMS (Sigma-Aldrich) as an internal standard. Mass spectra were obtained on JEOL-JMS-DX 303 instrument. Elemental analyses (C, H, N) were performed on Perkin-Elmer 240ºC analyzer. All compounds were within ±0.4% of the theoretical values. Thin layer chromatography was carried out using silica gel G-coated TLC plates (Merck).

Synthesis of target compounds

Synthetic protocol for N-(2-(1H-benzo[d]imidazol-2-yl) phenyl)-2-methylaniline (3)

1.26 g (0.01 M) of *o*-chlorotoluene and 0.3 M of diisopropylethylamine (DIPA) were dissolved in dry ethanol

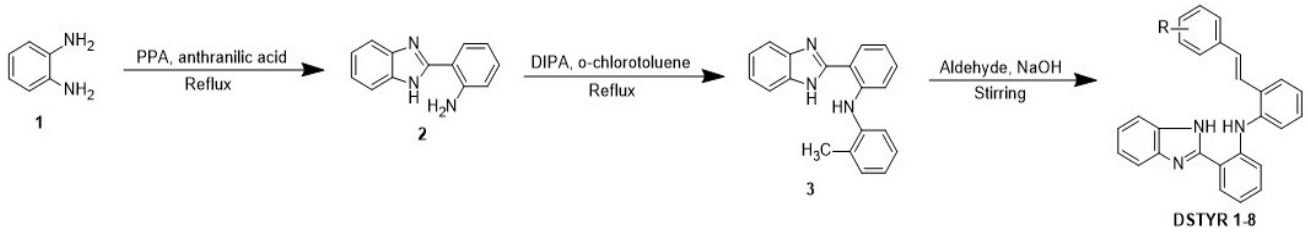


Figure 2: Synthetic protocol for synthesis of *(E)-N*-(2-(1*H*-benzo[d]imidazol-2-yl)phenyl)-2-(substituted-styryl)aniline derivatives. (1) DSTYR1: R = H; (2) DSTYR2: R = 2-Cl; (3) DSTYR3: R = 4-Cl; (4) DSTYR4: R = 4-F; (5) DSTYR5: R = 2-OH; (6) DSTYR6: R = 4-OH; (7) DSTYR7: R = 2-NO_2; and (8) DSTYR8: R = 4-OCH_3.

and added to 2.09 g (0.01 M) of 2-(1*H*-benzo[d]imidazol-2-yl)aniline (2). The content was refluxed in a closed steel vessel for 12 h. The solvent was removed under vacuum. To the remaining residue 50 mL of chloroform was added and stirred till the solution was affected. The organic layer was washed with water and dried over sodium sulfate. The crude product was precipitated by concentrating the solution and was then purified by recrystallization (Figure 2).

Synthetic protocol for (E)-N-(2-(1H-benzo[d]imidazol-2-yl)phenyl)-2-(substituted-styryl)aniline (DYSTR 1-8)

Equimolar quantities of different aldehydes and N-(2-(1*H*-benzo[d]imidazol-2-yl)phenyl)-2-methylaniline (3) were taken in a 250 mL round bottomed flask and dissolved in 20 mL of ethanol. Further, 10 mL of 20% sodium hydroxide solution was added and the mixture was stirred for 5 h. The reaction mixture was cooled to room temperature and neutralized by adding cold dilute hydrochloric acid drop wise. The obtained product was filtered under vacuum and dried suitably.

N-(2-(1*H*-benzo[d]imidazol-2-yl)-phenyl)-2-styryl)aniline (DSTYR1)

m.p. 112-114°C; yield: 66%, Rf: 0.36 [Benzene: Ethylacetate (9:1)]; IR: 3345.23 (N-H str), 3109.04 (aromatic C-H str), 1643.76 (aromatic C=C str), 1613.62 (C=C str of alkenes); ^{1}H-NMR (δ ppm, $CDCl_3$): δ 4.1 (s, 1H, N-H of benzimidazole ring), δ 6.9-8.1 (m, 17H, Ar-H) δ 5.1 (s, 1H, N-H, benzamine), δ 6.8 (d, 1H, vinylic), δ 6.5 (d, 1H, vinylic); MS (m/e): 387 [M+]; Elemental analysis (%) found (calculated): C 83.45 (83.69), H 5.36 (5.46), N 10.71 (10.84).

N-(2-(1*H*-benzo[d]imidazol-2-yl)phenyl)-2-(2-chlorostyryl)aniline (DSTYR2)

m.p. 78-80°C; yield: 60%; Rf: 0.42 [Benzene: Ethylacetate (9:1)]; IR: 3326.91 (N-H str), 3163.52 (aromatic C-H str), 1621.11 (aromatic C=C str), 1600.32 (C=C str alkenes); ^{1}H-NMR (δ ppm, $CDCl_3$): δ 4.4 (s, 1H, N-H of benzimidazole ring), δ 6.9-8.5 (m, 16H, Ar-H) δ 4.5 (s, 1H, N-H of benzamine), δ 6.9 (d, 1H, vinylic), δ 6.5 (d, 1H, vinylic); MS (m/e): 421 [M+]; Elemental analysis (%) found (calculated): C 76.67 (76.86), H 4.37 (4.78), N 9.83 (9.96).

N-(2-(1*H*-benzo[d]imidazol-2-yl)phenyl)-2-(4-chlorostyryl)aniline (DSTYR3)

m.p. 198-200°C; yield: 74%; Rf: 0.59 [Benzene: Ethylacetate (9:1)]; IR: 3385.01 (N-H str) 3022.04 (aromatic C-H str), 1639.42 (aromatic C=C str), 1602.00 (C=C str of alkenes); ^{1}H-NMR (δ ppm, $CDCl_3$): δ 4.0 (s, 1H, N-H ring), δ 6.8-7.7 (m, 16H, Ar-H) δ 4.5 (s, 1H, N-H of benzamine), δ 6.2 (d, 1H, vinylic), δ 6.5 (d, 1H, vinylic); MS (m/e): 421 [M+]; Elemental analysis (%) found (calculated): C 76.72 (76.86), H 4.69 (4.78), N 9.72 (9.96).

N-(2-(1*H*-benzo[d]imidazol-2-yl)phenyl)-2-(4-fluorostyryl)aniline (DSTRY4)

m.p. 244-246°C; yield: 65%; Rf: 0.51 [Chloroform: Methanol (9:1)]; IR: 3340.02 (N-H str), 3174.02 (aromatic C-H str), 1624.09 (aromatic C=C str), 1610.29 (C=C str of alkenes); ^{1}H-NMR (δ ppm, $CDCl_3$): δ 3.7 (s, 1H, N-H of benzimidazole ring), δ 6.6-7.9 (m, 16H, Ar-H) δ 4.2 (s, 1H, N-H of benzamine), δ 6.5 (d, 1H, vinylic), δ 6.6 (d, 1H, vinylic); MS (m/e): 405 [M+]; Elemental analysis (%) found (calculated): C 79.89 (79.98), H 4.92 (4.97), N 10.27 (10.36).

N-(2-(1*H*-benzo[d]imidazol-2-yl)phenyl)-2-(2-hydroxystyryl)aniline (DSTYR5)

m.p. 66-68°C; yield: 60%; Rf: 0.51 [Chloroform: Methanol (9:1)]; IR: 3283.82 (O-H str), 3034.08 (aromatic C-H str), 1629.62 (aromatic C=C str),1602.22 (C=C str of alkenes); ^{1}H-NMR (δ ppm, $CDCl_3$): δ 4.0 (s, 1H, N-H of benzimidazole ring), δ 6.6-8.3 (m, 16H, Ar-H) δ 4.2 (s, 1H, N-H of benzamine), δ 6.6 (d, 1H, vinylic), δ 6.9 (d, 1H, vinylic), δ 10.5 (s, 1H, Ar-OH); MS(m/e): 405 [M+]; Elemental analysis (%) found (calculated): C 80.31 (80.37), H 5.19 (5.25), N 10.31 (10.41).

N-(2-(1*H*-benzo[d]imidazol-2-yl)phenyl)-2-(4-hydroxystyryl)aniline (DSTYR6)

m.p. 78-80°C; yield: 70%; Rf: 0.56 [Chloroform: methanol (8:2)]; IR: 3283.82 (O-H str), 3034.08 (aromatic

C-H str), 1629.62 (aromatic C=C str),1602.22 (C=C str of alkenes); ^{1}H-NMR (δ ppm, $CDCl_3$): δ 4.2 (s, 1H, N-H of benzimidazole ring), δ 6.5-7.8 (m, 16H, Ar-H) δ 5.1 (s, 1H, N-H of benzamine), δ 6.6 (d, 1H, vinylic), δ 6.9 (d, 1H, vinylic), δ 8.9 (s, 1H, Ar-OH), MS (m/e): 403 [M+]; Elemental analysis (%) found (calculated): C 80.09 (80.37), H 5.18 (5.25), N 10.35 (10.41).

N-(2-(1*H*-benzo[d]imidazol-2-yl)phenyl)-2-(2-nitrostyryl) aniline (DSTYR7)

m.p. 192-194°C; yield: 60%; Rf: 0.40 [Benzene: Ethyl-acetate (9:1)]; IR: 3305.34 (N-H str), 3109.04 (aromatic C-H str), 1633.00 (aromatic C=C str), 1613.62 (C=C str of alkenes), 1479.36 and 1507.37 (N=O str sym. and antisym. respectively); ^{1}H-NMR: (δ ppm, $CDCl_3$): δ 4.1 (s, 1H, N-H of benzimidazole ring), δ 6.5-7.8 (m, 16H, Ar-H) δ 5.1 (s, 1H, N-H of benzamine), δ 6.8 (d, 1H, vinylic), δ 6.9 (d, 1H, vinylic); MS (m/e): 434 [M+]; Elemental analysis (%) found (calculated): C 74.89 (74.98), H 4.58 (4.66), N 12.52 (12.95).

N-(2-(1*H*-benzo[d]imidazol-2-yl)phenyl)-2-(4-methoxystyryl)aniline (DSTYR8)

m.p. 264-266°C; yield: 60%; R*f*: 0.39 [Chloroform: Methanol (9:1)]; IR: 3460 (NH str), 3050.04 (Ar C-H str), 1602.62 (C=C str of alkenes), 1639.60 (Ar C=C str), 1203.32(C-O str,Aryl-O-C) ^{1}H-NMR (δ ppm, $CDCl_3$) δ 1.2 (s, 3H, Methoxy) δ 4.3 (s, 1H, N-H of ring), δ 6.5-8.2 (m, 16H, Ar-H) δ 4.9 (s, 1H, N-H of benzamine), δ 6.8 (d, 1H, vinylic), δ 6.9 (d, 1H, vinylic); MS (m/e): 419 [M+]; Elemental analysis (%) found (calculated): C 80.46 (80.55), H 5.49 (5.55), N 9.89 (10.06).

In silico molecular docking

The molecular docking of ligands into the 3-D X-ray structure of protein tyrosine kinase (PDB ID: 2J5F) was carried out using Molecular Design Suite (MDS) software package (v. 3.5). The ligand-protein complex was created based on the X-ray structure of proteins obtained from protein data bank. All compounds were constructed using Chem Draw Ultra v. 8.0 and the energy minimization was done using the Merck Molecular Force Field (MMFF) application. The work is initiated by keeping the program parameter to their default options and the docking was carried out by MDS into the 3D model of the catalytic site of the enzyme. The comparative docking experiments of designed compounds with known kinase inhibitor imatinib were performed. The Genetic Algorithm (GA) implemented in the MDS has been used to dock inhibitors into the catalytic site of the target.[21] The obtained results were assessed in terms of the binding score.

Anticancer screening by Propidium Iodide assay (PI assay)

The non-small cell lung cancer cell line (H460; ATCC: HTB177) and human colorectal cancer cell line (HCT116; ATCC: CCL-247) were procured from NCCS, Pune. All cell lines were grown in media RPMI1640 enriched with fetal bovine serum (10%). All cell lines were grown under physiological conditions, specifically at 37°C with 5% CO_2 and passed every 2-3 days. The cell lines were freeze at -20°C for 24 h during which the seed cells (at a density range of 1500-3000 cells/well) in 180 µL of culture medium in tissue-culture-grade 96 well plate are allowed to recover for 24 h in humidified 5% CO_2 incubator at 37 ± 1°C. After 24 h, 20 µL solutions (1, 3, 10, 30, 100, 300 and 1000 µM of synthesized compounds dissolved in DMSO not exceeding 0.5 % and in cell medium), was added to wells and incubated for 48 h in humidified 5% CO_2 at 37 ± 1°C. After incubation, the medium was removed from wells and washed with Phosphate Buffered Saline (PBS). About 100 µL of PI working solution (7 µg/mL per well) was added and plates were stored at –80°C overnight. After thawing, the fluorescence of plates was measured using the POLAR star optima plate reader at excitation 536 nm and emission 590 nm.[22]

The anti-cancer potentials of compounds were calculated using the formula given below:

$$\% \text{ Cytotoxicity} = \left\{1 - \frac{[\text{corrected RFU of sample}]}{\text{corrected RFU control}}\right\} \times 100$$

Corrected control = [Average RFU of control - Average RFU of blank]

Corrected sample = [Average RFU of sample - Average RFU of blank]

Where, RFU: Relative fluorescence unit; Control: Well containing cancerous cell and PI solution; Blank: Well containing only PI solution.

Statistical analysis

The results acquired from experiments were expressed as mean. The differences between the control and treated groups were tested for significance using ANOVA followed by Dunnett's *t*-test, with $P < 0.05$ were considered as significant.

RESULTS AND DISCUSSION

In silico docking

The docking analysis into the active site of enzyme tyrosine protein kinase reveals that compounds having

Table 1: Docking Score of the synthesized derivatives.

Compound	Dock Score
DSTYR1	-11.27
DSTYR2	-10.81
DSTYR3	-12.13
DSTYR4	-10.91
DSTYR5	-13.63
DSTYR6	-5.99
DSTYR7	-10.21
DSTYR8	-13.23
Standard	-13.48

docking score of -11.27 kcal/mol (DSTYR1), -10.81 kcal/mol (DSTYR2), -12.13 kcal/mol (DSTYR3), -10.91 kcal/mol (DSTYR4), -13.63 kcal/mol (DSTYR5) and -13.23 kcal/mol (DSTYR8), indicating affinity for the compounds with receptor protein kinase. DSTYR5 and DSTYR8 demonstrated the strongest affinity for the enzyme as compared to other compounds in the series. The low dock score for DSTYR5 compared to standard imatinib, indicated stronger affinity for protein kinase (Table 1).

Chemistry

The IR spectra demonstrated some important characteristics of the synthesized molecules. The stretching of the amides was predominantly characterized by peaks in range 3280-3385 cm^{-1}. Two prominent features of the aromatic ring were observed in the spectrum. An aromatic C-H stretching in the range 3050-3200 cm^{-1} and aromatic C=C stretching was identified in the range 1610-1650 cm^{-1}. The alkene (C=C) stretching was primarily observed in the range of 1600-1610 cm^{-1}. The ^{1}H NMR signified the prominent structural features. The amide proton appeared in the spectrum at two different places. The protons of benzimidazole were detected in the range 3.7-4.2 ppm, whereas the protons of benzamide were located at 4.2-5.1 ppm. The peaks observed at 6.6-8.5 ppm, represented the 16 aromatic protons. The vinylic proton appeared prominently in the range of 6.2-6.9 ppm. The mass spectra described the base peaks corresponding or similar to their exact molecular mass. In-depth analysis of the mass spectrum described few key distinguishing features. The m/z 117.2 presented the benzo[d]imidazole fragment; m/z 193.3 signified the 2-phenyl-1*H*-benzo[d]imidazole fragment; and m/z 194.1 represented the *(E)*-1-amine-2-styrylbenzene part, which is equivalent to their molecular mass (Figure 3). Several fragment peaks also appeared in the m/z range of 100-150. The elemental

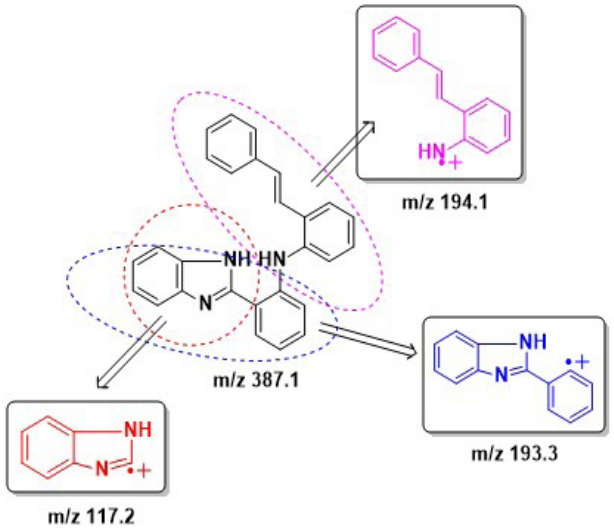


Figure 3: The fragmentation patterns of the prototype compound and the obtained products.

Table 2: IC_{50} values of synthesized derivatives against HCT116 and H460 cell lines.

Compound	IC_{50} (μM) against	
	HCT116	H460
DSTYR1	83.3	59.3
DSTYR2	95.5	262.7
DSTYR3	20.81	14.67
DSTYR4	2.98	5.15
DSTYR5	44.15	51.96
DSTYR6	57.5	52.25
DSTYR7	76.7	79.2
DSTYR8	39.9	41.13
Standard		

analysis of the compounds described the % of carbon, nitrogen and hydrogen which were found in close agreement with the theoretical value.

Anti-proliferative activity

The PI assay showed that compounds DSTYR3, DSTYR4, STYR5 and STYR8 exhibited tremendous cytotoxic activity against HCT116 and H460 cell lines (Table 2). The compounds DSTYR3 and DSTYR4 were found to be the potent candidate with IC_{50} values of 20.81 μM and 2.98 μM against HCT116 cell line and 14.67 μM and 5.15 μM against H460 cell line, respectively. The unsubstituted phenyl ring analog (DSTYR1) possess mild anticancer activity with IC_{50} of 59.3 μM. Based on the Structure Activity Relationship (SARs) and rational designing of benzimidazole scaffold, the substituents and their positions played an imperative role

in exhibiting anti-proliferative activity by modulating various unknown targets. The electron withdrawing substituents (Cl and F) results in an increase in anti-cancer potential with the IC_{50} value of 14.67 µM and 5.15 µM respectively for compounds DSTYR3 and DSTYR4 against H460. The fluoro analog (DSTYR4) having a log *P* value of 6.68, displayed the highest activity among all candidates, which might be indicative of the fact that the phenomenon of lipophilicity may be the driving factor. The lipophilicity (or hydrophobicity) of a compound is a crucial physical property that influences bilipid membrane permeation, dissolution rate, the bioavailability of compounds and controlling the interaction of drugs with the biological systems. Therefore, the candidate may interact with the target swiftly as compared to other analogs. From the observations, it was also noticed that analogs having electron withdrawing group in *para* position (DSTYR3) were privileged to express higher antiproliferative activity than *ortho* (DSTYR2); whereas the analogs having electron donating group in *ortho* position (DSTYR6) presented better activity than *para* (DSTYR5).

CONCLUSION

The study revealed the potential of *(E)*-N-(2-(1H-benzo[d]imidazol-2-yl)phenyl)-2-(substituted-styryl) aniline as active anti-proliferative candidates. The study reflected the profound role and positions of substitution on the phenyl moiety of the benzimidazole system. The compound DSTYR4 displayed most potent anti-proliferative activity with IC_{50} values of 2.98 µM against HCT116 cell line and 5.15 µM against H460 cell line. However, docking into the catalytic site of enzyme protein kinase revealed no true correlation between the cytotoxic activity data and the binding affinity of the compounds. It may be assumed that all the synthesized derivatives exerted their cytotoxic effect by any other mechanism, which has not been tested in the present work. Further, the work encourages medicinal chemists of rationally selecting benzimidazole prototypes having well defined MOA and SARs in designing more effective inhibitors. In addition, it opens new avenues of heterocyclic research for the development of anti-cancer derivatives. The research fruitfully rejuvenates the potentials and importance of small molecular weight ligands for experimental oncology.

ACKNOWLEDGEMENT

Authors sincerely acknowledge Board of College and University Development (BCUD) Savitribai Phule Pune University for providing research grants. We also acknowledge thanks to SAIF Chandigarh for undertaking spectroscopic analysis.

CONFLICT OF INTEREST

Authors have no conflict of interest with the content and publication of this article.

ABBREVIATIONS

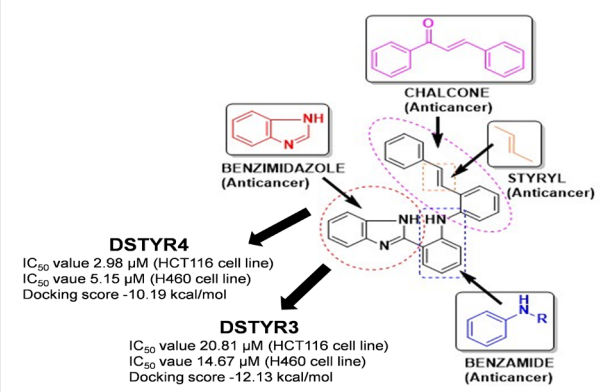
PBS: Phosphate Buffered Saline; **SAR:** Structure Activity Relationship; **LMWL:** Low molecular weight ligands; **RFU:** Relative Fluorescence Unit; **PI:** Propidium Iodide; **DIPA:** Diisopropylethylamine; **MDS:** Molecular Design Suite; **PDB:** Protein Data Bank; **MMFF:** Merck Molecular Force Field; **GA:** Genetic Algorithm; **MOA:** Mechanism of Action.

REFERENCES

1. http://www.who.int/mediacentre/factsheets/fs297/en/. 2018.
2. Chen K, Chu BZ, Liu F, Li B, Gao CM, Li LL, *et al*. New benzimidazole acridine derivative induces human colon cancer cell apoptosis *in vitro* via the ROS-JNK signaling pathway. Acta Pharmacol Sin. 2015;36(9):1074-84.
3. Soni LK, Narsinghani T, Sethi A. Anti-microbial benzimidazole derivatives: Synthesis and *in vitro* biological evaluation. Med Chem Res. 2012;21(12):4330-4.
4. Dighe S, Dighe N, Shinde PS, Lawre R, Nirmal S. Synthesis and Evaluation of Some New Benzimidazole Derivatives for their Anti-Microbial and Anti-Inflammatory Activities. Asian J Res Chem. 2014;7(12):1023-9.
5. Toro P, Klahn AH, Pradines B, Lahoz F, Pascual A, Biot C, *et al*. Organometallic benzimidazoles: Synthesis, characterization and antimalarial activity. Inorg Chem Commun. 2013;35:126-9.
6. Zhu W, Da Y, Wu D, Zheng H, Zhu L, Wang L, *et al*. Design, synthesis and biological evaluation of new 5-nitro benzimidazole derivatives as AT 1 antagonists with anti-hypertension activities. Bioorg Med Chem. 2014;22(7):2294-302.
7. Lemke TL, Williams DA. Foye's Principles of Medicinal Chemistry. Lippincott Williams and Wilkins. 2012.
8. Zheng Y, Zheng M, Ling X, Liu Y, Xue Y, An L, *et al*. Design, synthesis, quantum chemical studies and biological activity evaluation of pyrazole–benzimidazole derivatives as potent Aurora A/B kinase inhibitors. Bioorg Med Chem Lett. 2013;23(12):3523-30.
9. Maji B, Kumar K, Kaulage M, Muniyappa K, Bhattacharya S. Design and Synthesis of New Benzimidazole–Carbazole Conjugates for the Stabilization of Human Telomeric DNA, Telomerase Inhibition and their Selective Action on Cancer Cells. J Med Chem. 2014;57(16):6973-88.
10. Parajuli B, Fishel ML, Hurley TD. Selective ALDH3A1 inhibition by benzimidazole analogues increase mafosfamide sensitivity in cancer cells. J Med Chem. 2014;57(2):449-61.
11. Schäfer S, Ferioli S, Hofmann T, Zierler S, Gudermann T, Chubanov V. Mibefradil represents a new class of benzimidazole TRPM7 channel agonists. Pflügers Archiv Eur J Physiol. 2016;468(4):623-34.
12. Nare B, Liu Z, Prichard RK, Georges E. Benzimidazoles, potent anti-mitotic drugs: Substrates for the P-glycoprotein transporter in multidrug-resistant cells. Biochemical Pharmacology. 1994;48(12):2215-22.
13. Kamal A, Nagaseshadri B, Nayak VL, Srinivasulu V, Sathish M, Kapure JS, *et al*. Synthesis and biological evaluation of benzimidazole–oxindole conjugates as microtubule-targeting agents. Bioorg Chem. 2015;63:72-84.
14. Sharma A, Luxami V, Paul K. Synthesis, single crystal and antitumor activities of benzimidazole–quinazoline hybrids. Bioorg Med Chem Lett. 2013;23(11):3288-94.

15. Paul K, Bindal S, Luxami V. Synthesis of new conjugated coumarin–benzimidazole hybrids and their anticancer activity. Bioorg Med Chem Lett. 2013;23(12):3667-72.
16. Kalirajan R, Rathore L, Jubie S, Gowramma B, Gomathy S, Sankar S, *et al*. Microwave assisted synthesis and biological evaluation of pyrazole derivatives of benzimidazoles. Indian J Pharm Edu Res. 2010;44(4):358-62.
17. Wagle S, Adhikari AV, Kumari NS. Synthesis of some new 4-styryltetrazolo [1, 5-a] quinoxaline and 1-substituted-4-styryl [1, 2, 4] triazolo [4, 3-a] quinoxaline derivatives as potent anticonvulsants. Eur J Med Chem. 2009;44(3):1135-43.
18. Mahapatra DK, Bharti SK, Asati V. Anti-cancer chalcones: Structural and molecular target perspectives. Eur J Med Chem. 2015;98:69-114.
19. Mottamal M, Zheng S, Huang TL, Wang G. Histone deacetylase inhibitors in clinical studies as templates for new anticancer agents. Molecules. 2015;20(3):3898-941.
20. Long Y, Yu M, Li P, Islam S, Goh AW, Kumarasiri M, *et al*. Synthesis and biological evaluation of heteroaryl styryl sulfone derivatives as anticancer agents. Bioorg Med Chem Lett. 2016;26(23):5674-8.
21. Asati V, Bajaj S, Mahapatra DK, Bharti SK. Molecular modeling studies of some thiazolidine-2, 4-dione derivatives as 15-PGDH inhibitors. Med Chem Res. 2016;25(1):94-108.
22. Dengler WA, Schulte J, Berger DP, Mertelsmann R, Fiebig HH. Development of a propidium iodide fluorescence assay for proliferation and cytotoxicity assays. Anti-cancer Drugs. 1995;6(4):522-32.

PICTORIAL ABSTRACT



SUMMARY

- A series of hybridized molecules of the prototype *(E)*-N-(2-(1*H*-benzo[d]imidazol-2-yl)phenyl)-2-(substituted-styryl)aniline which comprises of a benzimidazole function; along with a chalcone (or styryl) moiety linked by a benzamide was designed.
- The compounds were characterized by sophisticated analytical techniques (IR, NMR, Mass spectroscopy) and the structure was established.
- DSTYR3 was found to be a potent candidate with IC_{50} value of 20.81 µM against HCT116 cell line and IC_{50} value of 14.67 µM against H460 cell line.
- DSTYR4 was found to be the most potent candidate with IC_{50} values of 2.98 µM against HCT116 cell line and IC_{50} value of 5.15 µM against H460 cell line.
- Docking score of -11.27 (DSTYR1), -10.81 (DSTYR2), -12.13 (DSTYR3), -10.91 (DSTYR4), -13.63 (DSTYR5) and -13.23 (DSTYR8), indicating affinity for the compounds with receptor protein kinase.

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Cite this article: Chhajed SS, Gupta PP, Kshirsagar S, Tomar S, Mahapatra DK, Sundararajan R. N-(2-(1H-benzo[d] imidazol-2-yl)Phenyl)-2- (Substituted-styryl)Aniline as Anti-proliferative Agents: Rejuvenating the Importance of Low Molecular Weight Ligands in Oncotherapeutics. Indian J of Pharmaceutical Education and Research. 2020;54(2):432-9.