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LETTERS

Synthesis of novel imidazolo-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine hybrids as *in vitro* EGFR inhibitors

Jagan Mohan Reddy Velidandla, Shiva Kumar Koppula*

Department of Chemistry, GITAM deemed to be University, Hyderabad campus, Rudraram, Sangareddy, Hyderabad, Telangana, India

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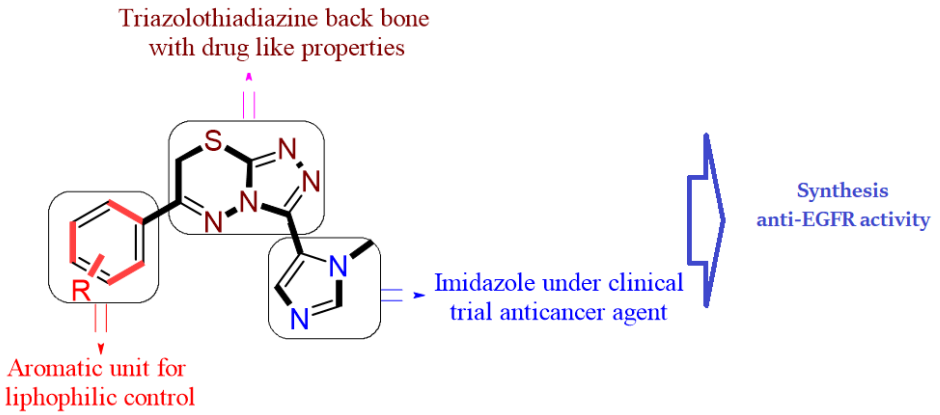
Article

ABSTRACT

The synthesis of new hybrid [1,2,4] triazolo [3,4-*b*][1,3,4]thiadiazine derivatives of imidazole (5a – 5m) and their structure determination using ^{1}HNMR, ^{13}CNMR and mass spectral analysis were described. The in vitro cytotoxic activity of the compounds (5a – 5m) against three human cancer cell lines like MCF-7 and MDA-MB-231 (breast), alveolar (A-549) revealed that the compounds 5c, 5d, 5f, 5g, and 5m have shown greater activity against breast cancer cell lines than the remaining compounds. Compounds 5d and 5f have shown equipotent activity compared to the standard. *In vitro* tyrosine kinase EGFR inhibition assay for the same more potent compounds (5c, 5d, 5f, 5g, and 5m) revealed that 5f has more potent inhibiting power with an IC_{50} value of 0.412±0.05 μM and 5d has equipotent inhibiting power with an IC_{50} value of 0.436±0.07 μM compared to erlotinib (IC_{50}=0.423±0.03).



Keywords: Imidazole; 1,2,4-triazole; thiadiazine; anticancer activity; EGFR

INTRODUCTION

Cancer is the second leading cause of death after cardiovascular diseases, accounting for a large number of deaths worldwide.[1] Protein kinases are the most studied druggable targets among the various anticancer drug targets known.[2] Because of its overexpression and elevation in multiple cancer subtypes, including non-small cell lung cancer (NSCLC), breast cancer, and ovarian cancer, the epidermal growth factor receptor (EGFR) belongs to the ErbB family of receptor tyrosine kinases.[3,4] The EGFR is thought to be the driver of cell proliferation, migration, adhesion, and survival, which leads to tumorigenesis via autophosphorylation via an intracellular signaling cascade.[5-7]

A review of the literature revealed that triazolothiadiazine and its derivatives, along with their diverse pharmacological activities, such as anticancer, antimicrobial, analgesic, and anti-inflammatory, antioxidant, antiviral, enzyme inhibitors, and antitubercular agents, are heterocycles.[8] This class of compounds' impressive anticancer activity is of particular interest. As a result, reports on the cytotoxic potency of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines against various cancer cell lines abound in the literature.[9-11] In the same way, imidazole and its derivatives are among the most important and widely used heterocycles in medicinal chemistry, natural products, and synthetic chemicals.[12] Because of the unique structural features of the imidazole ring, imidazole derivatives can easily attach to a variety of enzymes and receptors *via* a number of weak interactions, resulting in a variety of biological and pharmacological effects.[13] Imidazoles have been used as effective anticancer agents, among other pharmaceutical activities. Several imidazoles, such as dacarbazine, temzolomide, zoledronic acid, mercaptopurine, nilotinib, tipifarnib, and others, are currently being used in clinics to treat various cancers.[14]

Keeping all of the aforementioned facts in mind, as well as the benefits of pharmacophore hybridization,[15-17] we developed new hybrids with [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine linked imidazoles (Figure 1) and investigated their in vitro anticancer and tyrosine kinase EGFR inhibitory activity.

*Corresponding Author: Dr. Shiva Kumar Koppula
Department of Chemistry, GITAM deemed to be University, Hyderabad campus, Rudraram, Sangareddy, Hyderabad, 502329, Telangana, India.
*E-mail:- shivak.koppula78@gmail.com & skoppula@gitam.edu

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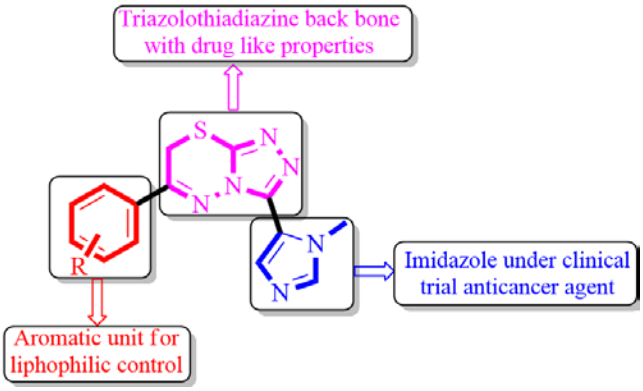
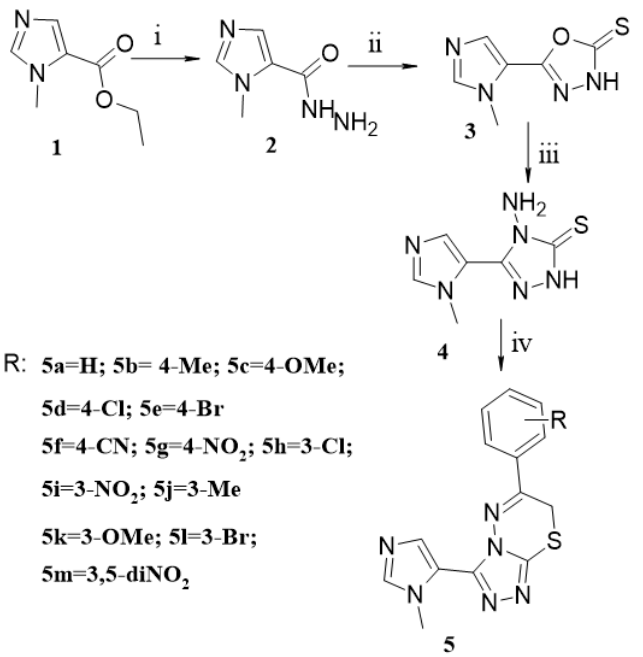


Figure 1: Design strategy for new imidazolo-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine hybrids.

RESULTS AND DISCUSSION

Chemistry

The synthesis of the title compounds is as outlined in Scheme 1. Ethyl 1-methyl-1*H*-imidazole-5-carboxylate (**1**) in reaction with hydrazine hydrate yields the corresponding 1-methyl-1H-imidazole-5-carbohydrazide (**2**).[18] Intramolecular cyclization of 2 with carbon disulfide resulted in 5-(1-methyl-1H-imidazol-5-yl)-1,3,4-oxadiazole-2(3H)-thione (**3**).[19] Compounds 3 were further treated with hydrazine hydrate to obtain compound 4-amino-3-(1-methyl-1H-imidazol-5-yl)-1H-1,2,4-triazole-5(4H)-thione (**4**).[20] The preparations of novel 3-(1-methyl-1H-imidazol-5-yl)-6-(aryl)-7H-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (**5a–5m**) was achieved with different phenacyl bromides.[21] The structures of the compounds were elucidated by NMR, mass, and elemental analysis.



Scheme 1: *Reagents & Conditions*. (i) NH_2-NH_2, EtOH, reflux, 2h; (ii) CS_2/KOH, EtOH, reflux, 8h & 2M HCl; (iii) NH_2-NH_2, EtOH, reflux, 6h; (iv) $ArCOCH_2Br$, EtOH, reflux, 5h & ether 0 ^oC, 12h.

All the spectral data of the synthesized compounds were in full agreement with the proposed structures and were also discussed for a representative compound, **5c**. In the ^{1}H-NMR spectrum, the signals are at δ 7.78–7.00 (Ar-H and Imidazole-H), 3.86 (s, 3H, OCH_3), 3.54 (s, 3H, CH_3), and 3.42 (s, 2H, -CH_2), and in the ^{13}C-NMR spectrum, the signals are at 159.32 (***C***-OCH_3), 56.35(-O***C***H_3), 34.56(-N***C***H_3), and 30.42(-S***C***H_2). The [M+H] ion peak at m/z 327.09 in the ESI mass spectrum confirmed the structure of compound **5c**. The elemental analysis (C, H, and N) data (C, 55.18; H, 4.34; N, 25.78) confirmed the purity of compound **5c**.

In vitro cytotoxicity activity

Later, all the new compounds (**5a–5m**) were further investigated for their *in vitro* cytotoxicity against three human cell lines, such as breast MCF-7 and MDA-MB-231 (breast), alveolar (A–549) and MCF-10 A (normal breast cell), using the MTT assay.[22] Erlotinib has been used as a positive control, and the results were accessible in IC_{50} with μM (Table 1, Figure 2). Those compounds demonstrated IC_{50} values ranging from 5.32 ± 0.65 μM to 44.69±1.13 μM, while the standard drugs displayed values ranging from 04.12 ± 0.11 to 10.39±0.76 μM respectively. Among the screened compounds, five compounds like 4-methoxy phenyl on thiadiazine ring (**5c**), 4-chloro phenyl on the thiadiazine ring (**5d**), 4-cyano phenyl on the thiadiazine ring (**5f**), 4-nitro phenyl on the thiadiazine ring (**5g**), and 3,5-dinitro phenyl on the thiadiazine ring (**5m**) were found to be active against two breast cancer cell lines. Predominantly, the compounds (**5f**) (MCF-7; IC_{50}= 5.32 ± 0.65 µM; & MDA-MB-231; IC_{50}= 08.12±0.71 μM) and (**5d**) (MCF-7; IC_{50}= 06.12 ± 0.41 μM; & MDA-MB-231; IC_{50}= 09.40± 0.86 μM) showed equipotent activity compared to the standard drug erlotinib.

In vitro EGFR tyrosine kinase inhibitory activity

The epidermal growth factor (EGFR) plays an important role in cell survival, growth, differentiation, and tumorigenesis. Accordingly, the inhibitory potential of a variety of imidazole, 1,2,4-triazole, and thiadiazine based derivatives against the tyrosine kinase EGFR was investigated.[23] In view of this, the inhibitory activity against the tyrosine kinase EGFR was tested for our five potent compounds (**5c**, **5d**, **5f**, **5g**, and **5m**), and the results were correlated with their *in vitro* cytotoxicity data. In detail, the compound **5f** (IC_{50}: 0.412±0.05 μM) showed superior activity than erlotinib (IC_{50}: 0.422±0.03 μM). Similarly, compound **5d** (IC_{50}: 0.436±0.07 µM) showed equipotent activity as compared to erlotinib, while the compounds **5m** (IC_{50}: 0.828±0.03 μM) showed good activity, and the remaining compounds **5c** (IC_{50}: 1.266±0.09 μM), and **5g** (IC_{50}: 1.156±0.07μM) displayed moderate EGFR inhibitory activity as compared to reference erlotinib.

EXPERIMENTAL SECTION

All reagents and solvents were obtained from commercial suppliers and used without further purification. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (GF 254) using UV light to visualize the course of the reactions. Melting points were determined using a Cintex apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker (operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C). Chemical

shifts (δ) are reported in ppm with TMS as the internal standard. Abbreviations for signal couplings are: *s*, singlet; *d*, doublet; *t*, triplet; *m*, multiplet. Routine monitoring of reactions was performed by TLC using 0.25 mm E. Merck precoated silica gel TLC plates (60 F254). Mass spectra were recorded on a Jeol JMC-300 spectrometer (ESI, 70 eV). Elemental analyses were performed on Carlo Erba 106 and Perkin-Elmer model 240 analyzers.

Table 1: *In vitro* cytotoxicity activity with IC_{50} in μM and EGFR inhibitory activity of potent compounds**.**

	IC_{50}(μM)[a]				
Compound	**MCF-7**	**MDA-MB-231**	**A-549**	**MCF-10 A**	**EGFR**
5a	33.81± 1.43	44.69±1.13	39.65 ± 1.11	NT	NT
5b	25.33± 1.12	38.66± 1.23	34.61± 1.27	NT	NT
5c	09.42± 0.78	12.34± 0.76	19.17 ± 1.67	28.34 ± 1.21	1.266±0.09
5d	06.12 ± 0.41	09.40± 0.86	18.38±1.22	25.13 ± 1.14	0.436±0.07
5e	15.32± 1.02	17.88±1.07	25.89±1.12	NT	NT
5f	5.32 ± 0.65	08.12±0.71	19.86 ± 1.01	24.53 ± 1.22	0.412±0.05
5g	8.66 ± 1.01	13.98±0.72	18.43± 0.98	21.29 ± 1.11	1.156±0.07
5h	10.89±0.71	15.65±0.62	20.43±1.02	NT	NT
5i	13.66± 0.89	17.39 ± 1.02	25.83±1.11	NT	NT
5j	34.66±1.08	37.65±1.19	41.69±1.01	NT	NT
5k	14.69±0.99	27.34±1.05	21.87±1.16	NT	NT
5l	12.87±1.11	25.83 ± 1.08	22.43±1.01	NT	NT
5m	06.71±0.82	11.45± 0.93	18.59±1.02	16.38 ± 1.08	0.828±0.03
Erlotinib	**04.12 ± 0.11**	**08.09±0.66**	**10.39±0.76**	NT	**0.423±0.03**

[a] Each data represents as mean ± S.D values.

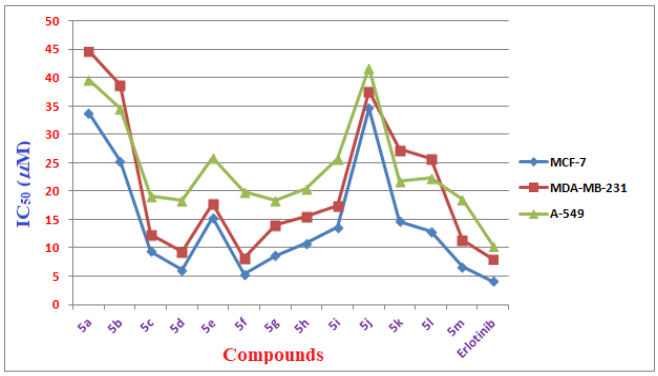


Figure 2: *In vitro* cytotoxicity activity against MCF-7, MDA-MB-231, and A-549 of compounds 5a-5m.

Synthesis of 1-methyl-1H-imidazole-5-carbohydrazide (2): To ethyl 1-methyl-1H-imidazole-5-carboxylate (1) (5 mmol) in methanol (20 ml), 80% hydrazine hydrate (5 mmol) was added in drop wise and the reaction mixture was refluxed about 2h. A white solid separated, which on recrystallization with ethanol gave 2 (78%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.10 (brs, 1H, -CONH), 7.75 (s, 1H, CH), 7.10 (s, 1H, CH), 4.21 (brs, 2H, $-NH_2$), 3.55 (s, 3H, CH_3); EI-MS m/z 141.07 [M+H].

Synthesis of 5-(1-methyl-1H-imidazol-5-yl)-1,3,4-oxadiazole-2(3H)-thione (3): To a solution of **2** (3.00 mmol) and carbon disulfide (6.00 mmol) in absolute ethanol (20 ml), potassium hydroxide (3.00 mmol) was added in one portion at 0 °C. The resulting mixture was stirred and refluxed for 8 hours. The solvent was removed, and the residue was acidified with 2M hydrochloric acid and extracted with ethyl acetate (3 × 30 ml). Organic layers were washed with water and dried with anhydrous sodium sulphate (Na_2SO_4). Filtration and concentration in vacuo gave a solid, which was recrystallized from ethanol to give 3 (74%) as pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.75 (brs, 1H, -NH), 7.73 (s, 1H, CH), 7.08 (s, 1H, CH), 3.53 (s, 3H, CH_3); ESI-MS m/z 183.03[M+H].

Synthesis of 4-amino-3-(1-methyl-1H-imidazol-5-yl)-1H-1,2,4-triazole-5(4H)-thione (4): To a mixture of 3 (3.00 mmol) in ethanol (20 ml), 0.4 ml of 30% hydrazine hydrate was added drop wise and the mixture was refluxed for 6h. After cooling water was added and the mixture was acidified by excess of 3M hydrochloric acid, the separated solid was filtered off, washed with water and crystallized from ethanol to give 4 (70 %) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.11 (brs, 1H, -CONH), 7.79 (s, 1H, CH), 7.12 (s, 1H, CH), 3.77 (brs, 2H, $-NH_2$), 3.52 (s, 3H, CH_3); ESI-MS m/z 197.05 [M+H].

General procedure for the synthesis of 3-(1-methyl-1H-imidazol-5-yl)-6-(aryl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5a-5m).

A mixture of 4 (1.50 mmol) and phenacyl bromide (1.50 mmol) in anhydrous ethanol (15 ml) was refluxed for 5h. The solvent was removed under reduced pressure, diethyl ether (20 ml) was added, and the reaction mixture was left at 0 °C overnight. The precipitated solid was filtered off, dried and recrystallized with ethanol to give the title compounds.

3-(1-methyl-1H-imidazol-5-yl)-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5a):

Pale red solid; mp 129-131 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H, CH), 7.68-7.63 (m, 3H, Ar-H), 7.50-7.43 (m, 2H, Ar-H), 7.12 (s, 1H, CH), 3.52 (s, 3H, CH_3), 3.42 (s, 2H, $-CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.60, 150.28, 140.53, 135.06, 134.40, 129.92, 128.52(2C), 126.65(2C), 121.22, 111.73, 34.62, 30.55; ESI-MS m/z: 297.08 [M+H]. Anal.Cal for $C_{14}H_{12}N_6S$; C, 56.74; H, 4.08; N, 28.36; found C, 56.76; H, 4.05; N, 28.39.

3-(1-methyl-1H-imidazol-5-yl)-6-(p-tolyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5b): Red solid; mp 134-136 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (s, 1H, CH), 7.60 (d, *J*= 8.0 Hz, 2H, Ar-H), 7.42 (d, *J*= 8.0 Hz, 2H, Ar-H), 7.11 (s, 1H, CH), 3.54 (s, 3H, CH_3), 3.41 (s, 2H, $-CH_2$), 2.33 (s, 3H, Ar-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.47, 150.31,

140.25, 139.52, 135.56, 133.24, 128.77(2C), 127.62(2C), 121.29, 111.25, 34.61, 30.87, 21.31; ESI-MS m/z: 311.10 [M+H]. Anal.Cal for $C_{15}H_{14}N_6S$; C, 58.05; H, 4.55; N, 27.08; found C, 58.06; H, 4.52; N, 28.01.

6-(4-methoxyphenyl)-3-(1-methyl-1H-imidazol-5-yl)-7H-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazine (5c): Pale red solid; mp 145-147 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (s, 1H, CH), 7.71 (d, *J*= 8.0 Hz, 2H, Ar-H), 7.12 (s, 1H, CH), 7.00 (d, *J*= 8.0 Hz, 2H, Ar-H), 3.86 (s, 3H, OCH_3), 3.54 (s, 3H, CH_3), 3.42 (s, 2H, -CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.32, 151.53, 150.39, 140.49, 135.69, 129.46, 128.12(2C), 121.18, 114.28(2C), 111.29, 56.35, 34.56, 30.42; ESI-MS m/z 327.09 [M+H]; Anal. Cal for $C_{15}H_{14}N_6OS$: C, 55.20; H, 4.32; N, 25.75; found C, 55.18; H, 4.34; N, 25.78.

6-(4-chlorophenyl)-3-(1-methyl-1H-imidazol-5-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5d): Pale red solid; mp 140-142 °C; ^{1}H NMR (400 MHz, $CDCl_3$): 7.79 (s, 1H, CH), 7.71 (d, *J*= 8.0 Hz, 2H, Ar-H), 7.52 (d, *J*= 8.0 Hz, 2H, Ar-H), 7.13 (s, 1H, CH), 3.55 (s, 3H, CH_3), 3.43 (s, 2H, -CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.67, 150.38, 140.53, 137.50, 135.49, 134.42, 129.58(2C), 127.78(2C), 121.28, 111.19, 34.21, 30.60; ESI-MS m/z 331.05 [M+H].; Anal. Cal for $C_{14}H_{11}ClN_6S$: C, 50.83; H, 3.35; N, 25.41; found C, 50.80; H, 3.32; N, 25.44.

6-(4-bromophenyl)-3-(1-methyl-1H-imidazol-5-yl)-7H-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazine (5e): White solid; mp 158-160 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H, CH), 7.66-7.58 (m, 4H, Ar-H), 7.10 (s, 1H, CH), 3.54 (s, 3H, CH_3), 3.41 (s, 2H, -CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.62, 150.28, 140.67, 135.37, 133.87, 132.19(2C), 128.83(2C), 125.53, 121.14, 111.52, 34.39, 30.44.; ESI-MS m/z: 374.99 [M+H] & 375.99 [M+H]. Anal. Cal for $C_{14}H_{11}BrN_6S$: C, 44.81; H, 2.95; N, 22.40; found: C, 44.83; H, 2.91; N, 22.44.

4-(3-(1-methyl-1H-imidazol-5-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6-yl) benzonitrile *(5f):* White solid; mp 152-154 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H, CH), 7.68 (d, *J*= 8.0 Hz, 2H, Ar-H), 7.48 (d, *J*= 8.0 Hz, 2H, Ar-H), 7.13 (s, 1H, CH), 3.54 (s, 3H, CH_3), 3.44 (s, 2H, -CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.68, 150.29, 140.65, 136.85, 135.04, 132.81(2C), 130.60(2C), 121.14, 118.81, 116.64, 111.27, 34.48, 30.69.; ESI-MS m/z: 322.08 [M+H]. Anal. Cal for $C_{15}H_{11}N_7S$: C, 56.06; H, 3.45; N, 30.51; found: C, 56.08; H, 3.42; N, 30.55.

3-(1-methyl-1H-imidazol-5-yl)-6-(4-nitrophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5g): Pale yellow solid; mp 162-164 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, *J*= 8.0 Hz, 2H, Ar-H), 8.25 (d, *J*= 8.0 Hz, 2H, Ar-H), 7.80 (s, 1H, CH), 7.13 (s, 1H, CH), 3.56 (s, 3H, CH_3), 3.44 (s, 2H, -CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.77, 150.42, 148.21, 142.80, 140.40, 135.53, 128.84(2C), 125.72(2C), 121.06, 111.49, 34.57, 30.58.; ESI-MS m/z: 342.07 [M+H]. Anal.Cal for $C_{14}H_{11}N_7O_2S$; C, 49.26; H, 3.25; N, 28.72; found C, 49.23; H, 3.22; N, 28.76.

6-(3-chlorophenyl)-3-(1-methyl-1H-imidazol-5-yl)-7H-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazine (5h): White solid; mp 138-140 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H, CH), 7.69 (s, 1H, CH), 7.55-7.45 (m, 3H, Ar-H), 7.13 (s, 1H, CH), 3.53 (s, 3H, CH_3), 3.43 (s, 2H, -CH_2); ESI-MS m/z: 331.05 [M+H]. Anal. Cal for $C_{14}H_{11}ClN_6S$; C, 50.83; H, 3.35; N, 25.41; found C, 50.80; H, 3.31; N, 25.43.

3-(1-methyl-1H-imidazol-5-yl)-6-(3-nitrophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5i): Pale red solid; mp 153-155 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (s, 1H, CH), 8.30 (d, *J*= 4.0 Hz, 1H, Ar-H), 7.90-7.84 (m, 2H, Ar-H), 7.76 (s, 1H, CH), 7.11 (s, 1H, CH), 3.53 (s, 3H, CH_3), 3.43 (s, 2H, -CH_2); ESI-MS m/z: 342.07 [M+H]. Anal.Cal for $C_{14}H_{11}N_7O_2S$; C, 49.26; H, 3.25; N, 28.72; found C, 49.24; H, 3.21; N, 28.75.

3-(1-methyl-1H-imidazol-5-yl)-6-(m-tolyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5j): Pale yellow solid; mp 122-124 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H, CH), 7.64-7.60 (m, 3H, Ar-H), 7.43 (s, 1H, Ar-H), 7.10 (s, 1H, CH), 3.53 (s, 3H, CH_3), 3.43 (s, 2H, -CH_2), 2.37 (s, 3H, Ar-CH_3); ESI-MS m/z: 311.10 [M+H].Anal. Cal for $C_{15}H_{14}N_6S$: C, 58.05; H, 4.55; N, 27.08; found: C, 58.02; H, 4.58; N, 27.09.

6-(3-methoxyphenyl)-3-(1-methyl-1H-imidazol-5-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5k): Yellow solid; mp 136-138 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, 1H, CH), 7.69-7.63 (m, 2H, Ar-H), 7.50-7.46 (m, 1H, Ar-H), 7.13 (s, 1H, CH), 7.01 (s, 1H, ArH), 3.85 (s, 3H, OCH_3), 3.52 (s, 3H, CH_3), 3.41 (s, 2H, -CH_2); ESI-MS m/z: 327.09 [M+H]. Anal.Calfor $C_{15}H_{14}N_6OS$; C, 55.20; H, 4.32; N, 25.75; found C, 55.21; H, 4.35; N, 25.79.

6-(3-bromophenyl)-3-(1-methyl-1H-imidazol-5-yl)-7H-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazine (5l)*:* White solid; mp 150-152 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H, CH), 7.69 (s, 1H, Ar-H), 7.60-7.53 (m, 3H, Ar-H), 7.11 (s, 1H, CH), 3.53 (s, 3H, CH_3), 3.42 (s, 2H, -CH_2); ESI-MS m/z: 374.99 [M+H] & 376.99 [M+3H]. Anal. Cal for $C_{14}H_{11}BrN_6S$: C, 44.81; H, 2.95; N, 22.40; found: C, 44.76; H, 2.92; N, 22.43.

6-(3,5-dinitrophenyl)-3-(1-methyl-1H-imidazol-5-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5m)*:* White solid; mp 170-172 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 1H, Ar-H), 8.30 (s, 2H, Ar-H), 7.81 (s, 1H, CH), 7.13 (s, 1H, CH), 3.54 (s, 3H, CH_3), 3.43 (s, 2H, -CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.60, 150.29, 146.86(2C), 140.69, 138.50, 135.32, 126.11(2C), 124.15, 121.38, 111.30, 34.63, 30.52.; ESI-MS m/z: 387.05 [M+H]. Anal. Cal for $C_{14}H_{10}N_8O_4S$: C, 43.52; H, 2.61; N, 29.00; found: C, 43.47; H, 2.56; N, 29.08.

Conclusion

In conclusion, we have synthesized some novel hybrid [1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**5a–5m**) using previously reported methods. All the newly synthesized compounds screened for their anticancer activity against three cancer cell lines MCF-7, MDA-MB-231, and A-549. Amongst the series, compounds **5c**, **5d**, **5f**, **5g**, and **5m** displayed higher *in vitro* cytotoxic activity against the tested breast cancer cell lines (MCF-7, and MDA-MB-231) than the remaining compounds. Besides, more potent compounds further evaluated enzyme inhibition activity against tyrosine kinase EGFR protein, while, compound **5f** showed superior activity and compound **5d** showed equipotent activity as compared to erlotinib.

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CONFLICT OF INTEREST STATEMENT

Authors declare that there is no academic or financial conflict of interest for publication of this work.

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