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Design, synthesis and anti-tumour activity of new pyrimidine-pyrrole appended triazoles

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ABSTRACT

The new pyrimidine-pyrrole scaffolds (**7a–7m**) with substituted 1,2,3-traizole moiety were synthesized in good to mild yields and subjected for anti-cancer activity against melanoma and breast cancer cell lines using MTT assay. The compounds **7f** and **7m** exhibited highest anti-cancer activity against both the tested cell lines in *in vitro* assay. The molecular docking analysis provided the insights of binding orientation of pyrimidine-pyrrole nucleus of current ligands and their crucial interactions with Cys797 and other residues of the EGFR tyrosine kinase active site. The interactions of triazole and its various substituted groups with EGFR tyrosine kinase have been discussed.

1. Introduction

The proliferative cancer disease is affecting the individuals in many forms across the globe. The concept of small therapeutic agents to fight against cancer has attained much popularity in cancer drug discovery (Hoelder et al., 2012). The mutations in several cancers due to environmental, lack of physical activity and infectious conditions make treatment difficulty with existing drugs (Parsa, 2012). Pyrimidine nucleus is an integral part of DNA, RNA and plays a vital role in several biological processes (Esteban-Gamboa et al., 2000a, 2000b). 1,2-diazine (pyridazine) and 1,4-diazine (pyrazine) are two isomeric forms of pyrimidine (Mansour et al., 2003). Pyrimidine derivatives received great attention due to their broad spectrum of biological and pharmacological activities such as anticancer (Reynolds et al., 2000; Iyer et al., 2000; Parveen et al., 2010; Focher et al., 2000), antiviral (Kraljević et al., 2012; Choi et al., 2000), anti-HIV (Hopkins et al., 1999; Pontikis et al., 2000), antimicrobial (Chowdhury et al., 1999), anti-inflammatory (Ohmoto et al., 2000), platelet aggregation inhibitors and anti-parkinsonism agents (Al-Harbi et al., 2013). The pyrimidine core is a structural constituent of vital biomolecules like thiamine, riboflavin and folic acid (Li et al., 2006; Xi et al., 2006). During the last two decades, numerous pyrimidine derivatives have been developed as chemotherapeutic agents employing it as an important structural element. The pyrimidine based anti-cancer agents like fluorouracil, cepacitabine, cytarabine, gemcitabine, bleomycin and cladribine are employed as medications against most of the cancers. Numerous recent reports also highlighted the anticancer potential of pyrimidines in fused scaffolds (Temburnikar et al., 2014; Dwyer et al., 2011; Kamal et al., 2013; Yu et al., 2013). Pyrrole is an important five membered nitrogen containing heterocycle, found in many natural and synthetic drugs (Sternberg et al., 1998; Melvin et al., 2000; Fürstner, 2003), which are employed for activities like anti-cancer, antifungal, cyclooxygenase inhibitor, antianxiety and antidiabetic (Kashman et al., 1999; Jouanneau et al., 2016; Tafi et al., 2002; Di Santo et al., 2005; Khanna et al., 1997; Olgen et al., 2001; Li et al., 2016a, 2016b). The new pyrrolo[3,2-*d*]pyrimidine analogs were investigated as significant anti-proliferative agents against a set of cancer cells (Temburnikar et al., 2015).

A series of fused pyrrole analogues include pyrazolopyrrolopyrimidine, triazinopyrrolopyrimidine, pyrrolopyrimidotriazepines, pyrrolopyrimidines, triazolopyrrolopyrimidines, and tetrazolopyrrolopyrimidine exhibited excellent cytotoxic activity (Ghorab et al., 2014). Simultaneously, most of the drugs like anticancer molecule –CAI, non-nucleoside reverse transcriptase inhibitor-TSAO, CNS-active

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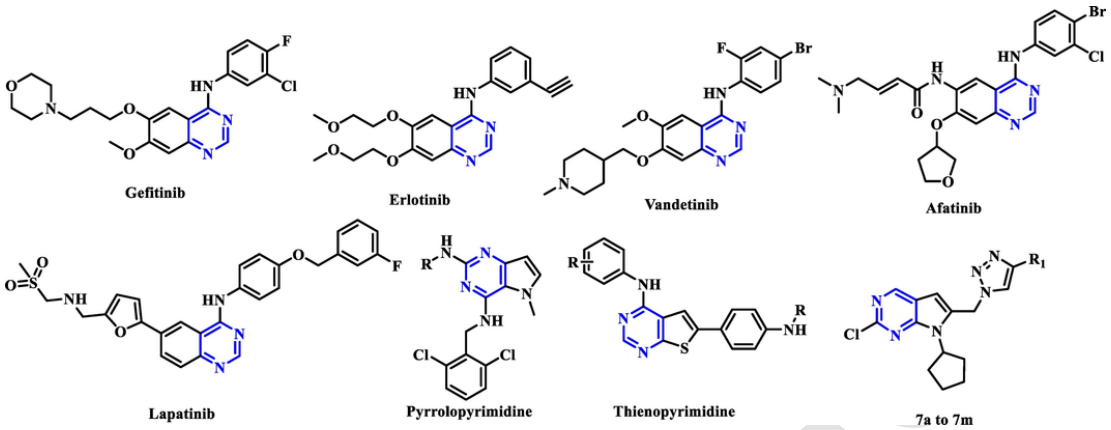
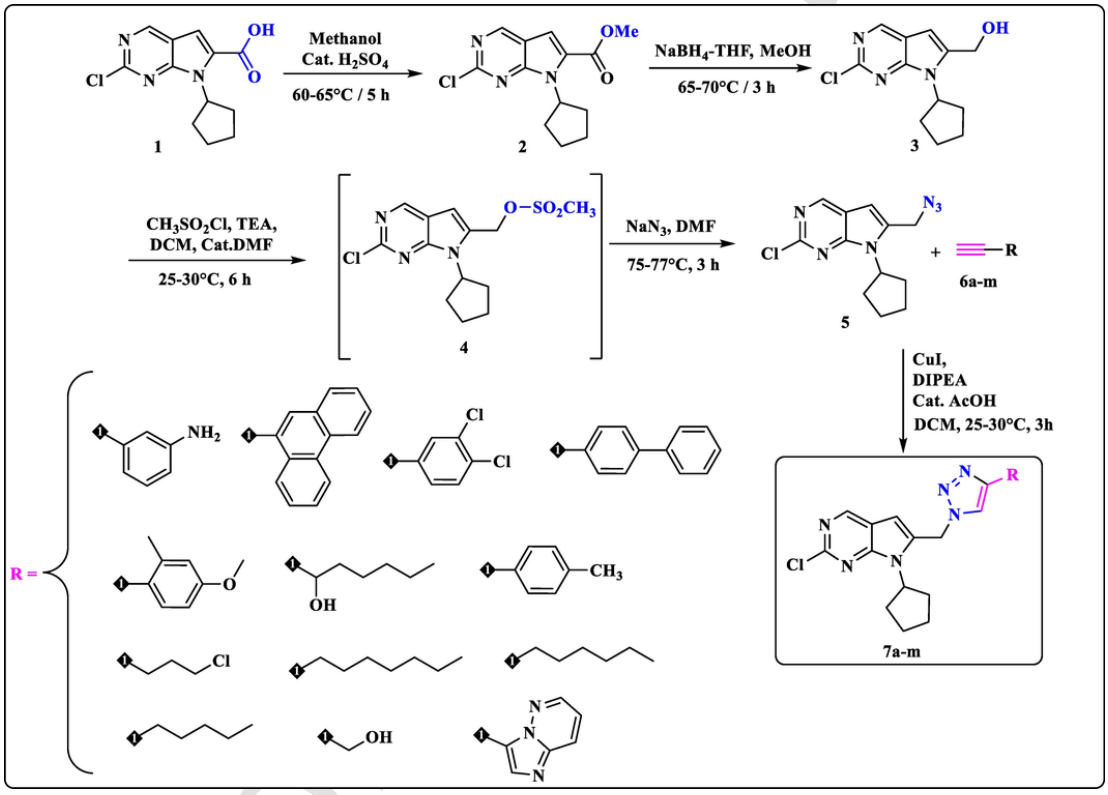


Fig. 1. Pyrimidine based anti-cancer agents.



Scheme 1. Synthesis of pyrimidine-pyrrole appended substituted triazole derivatives **(7a–m)**

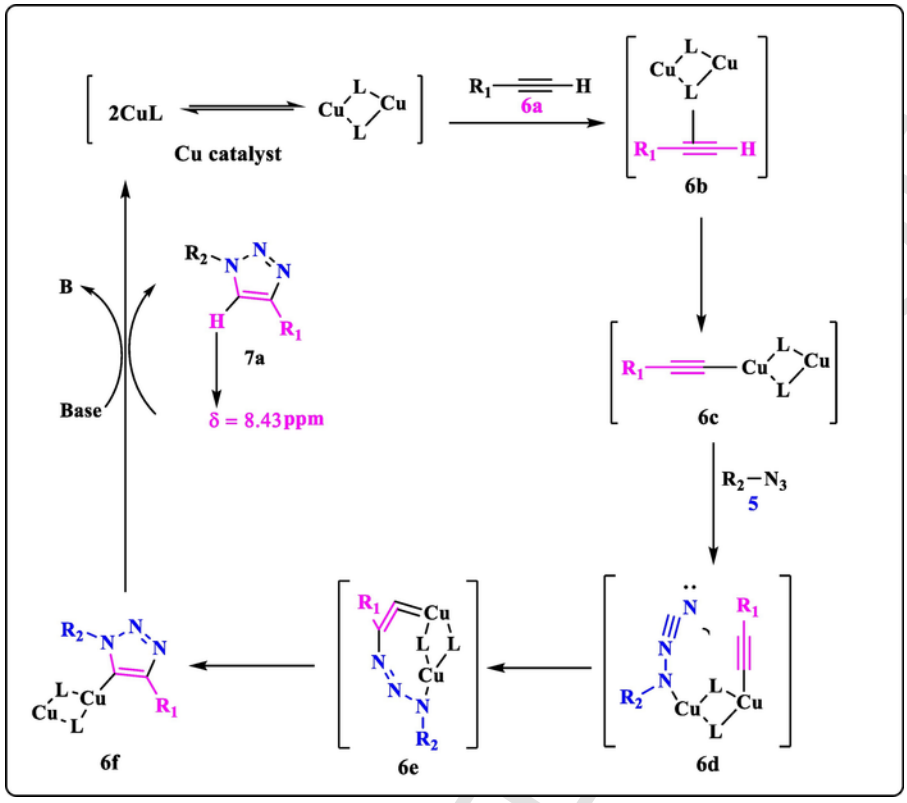
compound-Rufinamide, Cefatrizine and β-lactum antibiotic-Tazobactum were derived from the triazoles (Soltis et al., 1996; Sheng and Zhang, 2011). The combined therapeutic potentials of 1,2,3- triazole scaffolds have accomplished the great significance in the field of medicinal chemistry (Sheng and Zhang, 2011). The effort to develop new small target specific (EGFR Tyrosine kinases) pyrimidine derived anti-cancer agents, provided FDA approved drugs like gefitinib, erlotinib, vandetinib, afatinib and lapatinib (Li et al., 2016a, 2016b; Milik et al., 2018). The pyrimidine based agents such as thienopyrimidine and pyrrolopyrimidine derivatives were studied as effective anti-cancer agents and EGFR inhibitors (EP3290420A1) (Fig. 1).

Confident by the aforementioned statements on pyrimidine, its allied agents and triazole based anti-cancer agents; a series of pyrrolopyrimidine based triazoles were rationally sketched and synthesized in good to moderate yields. The newly synthesized pyrrolopyrimidine based triazoles were subjected to anti-cancer screening against few cancer cell lines and their probable binding modes in EGFR tyrosine kinase have been carried out using molecular docking investigations (Milik et al., 2018; Mule et al., 2016; Ahsan et al., 2013).

2. Materials and methods

2.1. Reagents and synthetic procedures

All the chemicals were obtained from commercial sources and used without further purification. Melting points were determined in open glass capillaries on a Fisher–Johns melting point apparatus and are uncorrected. NMR (^{1}H 400 MHz; ^{13}C 100 MHz) spectra were recorded at room temperature in DMSO-d_6 and $CDCl_3$ as solvent and TMS as an internal standard (δ = 0 ppm), and the values were reported in the following order: chemical shift (δ in ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, qq = quartet of



Scheme 2. The plausible mechanism of 1,3-dipolar cycloaddition for triazole derivative **7a**

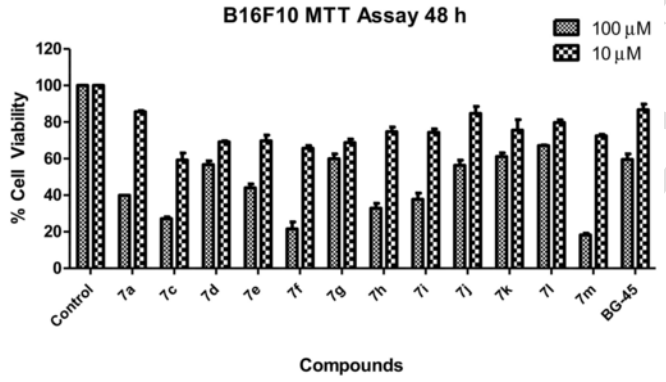


Fig. 2. Antiproliferative activity of all novel compounds from triazoles series by MTT assay. Compounds were tested for cytotoxicity on B16F10 cells (Murine Melanoma cell line) for 48 h. BG45 was used as positive control, Data presented here are mean ± SD (n = 3).

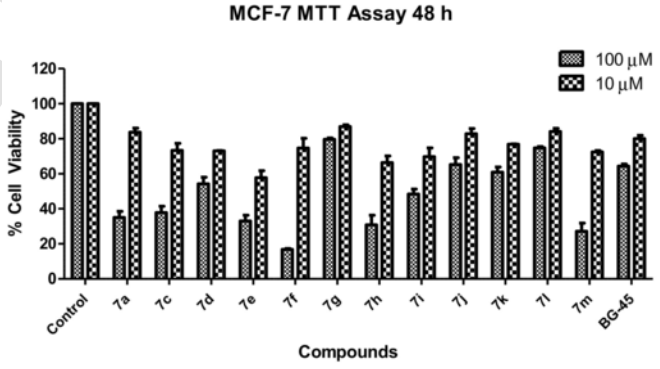


Fig. 3. Antiproliferative activity of all novel compounds from triazoles series by MTT assay. Compounds were tested for cytotoxicity on MCF-7 cells (Breast Cancer cell line) for 48 h. BG45 was used as positive control for the anticancer assay. Data presented with mean ± SD (n = 3).

quartet), coupling constants (J in Hz), and integration. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F254 (mesh); spots were visualized under UV light at 254 nm.

2.1.1. Synthetic procedure for compound 2

To the stirred solution of methanol (100.0 mL) and 2-chloro-7-cyclopentyl-7H-pyrrolo[2,3-*d*]pyrimidine-6-carboxylic acid **1** (10.0 g, 0.037 mol), Conc.sulfuric acid (H_2SO_4) (0.18 g, 0.05 mol) was added at 5–10 °C and heated at 60–65 °C for 3.0 h. Reaction completion was confirmed by TLC (solvent) and distilled off the solvent completely under vacuum at 45–50 °C to get the residual mass. Residual mass was dissolved in Dichloromethane (100.0 mL) and washed with 5% Aq. Sodium bicarbonate ($NaHCO_3$) solution (50.0 mL) followed by demineralized water (DM Water) (50.0 mL). The separated organic layer dried over anhydrous sodium sulfate (Na_2SO_4). The obtained organic layer distilled off and isolated from n-heptane (50.0 mL), to give ester **2** as a creamy solid 8.3 g (79%).

2.1.2. Synthetic procedure for compound 3

A stirred solution of tetrahydrofuran (80.0 mL), methanol (12.0 mL) and methyl 2-chloro-7-cyclopentyl-7H-pyrrolo[2,3-*d*]pyrimidine-6-carboxylate **2** (8.0 g, 0.028 mol) was charged with sodium borohydride ($NaBH_4$)(2.1 g, 0.056 mol) at 25–30 °C. Heated the reaction mass to 60–65 °C and stirred for 3.0 h. Reaction completion was monitored by TLC and quenched with aqueous saturated ammonium chloride (NH_4Cl) (20.0 mL). Product was extracted in to ethyl acetate ($CH_3COOC_2H_5$) (160.0 mL) and separated the layers. The separated organic layer was washed demineralized water (DM water) (50.0 mL) and dried over anhydrous sodium sulfate (Na_2SO_4). The obtained or-

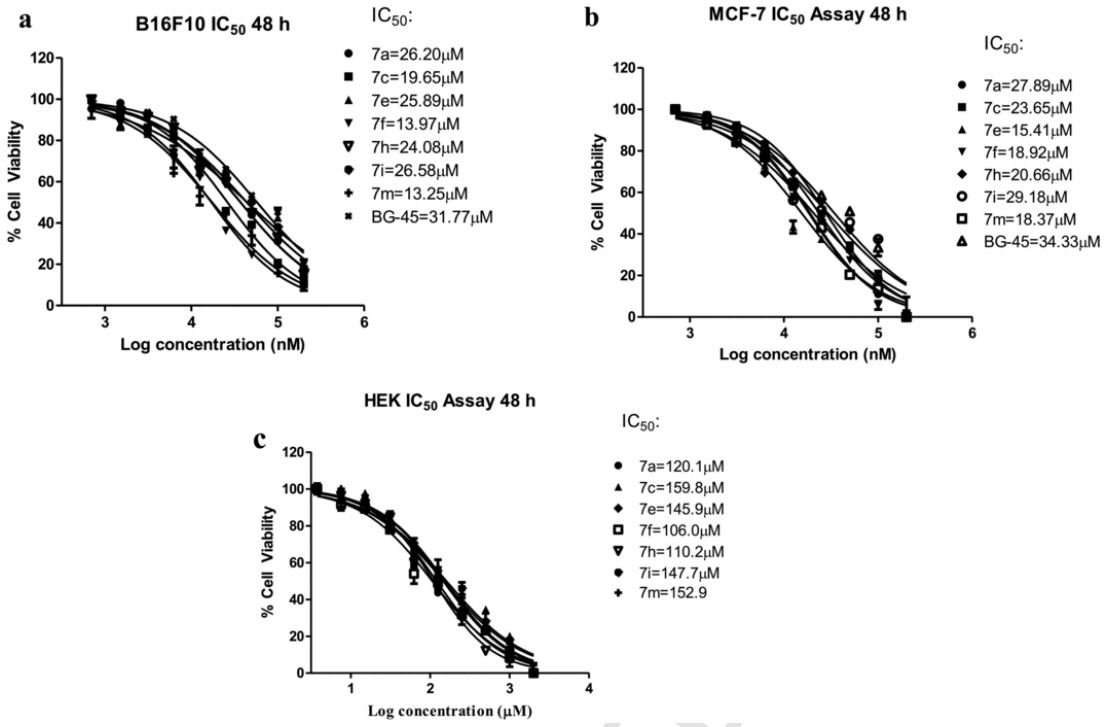


Fig. 4. 4a&4b: The promising compounds from triazoles series were screened further to find out their IC_{50} values. **7a, 7c, 7e, 7f, 7h, 7i** and **7m** were evaluated in ten different doses on B16F10 and MCF-7 cell lines for 48 h and cell viability was measured using MTT assay. BG45 was used as positive control and data presented as mean ± SD ($n = 2$). 4c: The promising compounds from triazole series were screened further to find out their cytotoxic IC_{50} values with noncancerous normal cell lines. 7a, 7c, 7e, 7f, 7h, 7i and 7m were evaluated in ten different doses on human embryonic kidney cells (HEK-293) for 48 h and cell viability was measured using MTT assay.

Table 1
Anti-cancer activity data (IC_{50} values) and docking scores of **7a**–**m**.

S. No.	Entry	IC_{50} Value (μM)		Docking score Kcal/mol	Binding free energy MM-GBSA
		B16F10	MCF-7		
1	**7a**	26.20	27.89	−6.049	−59.53
2	**7c**	19.65	23.69	−6.171	−62.66
3	**7d**	>50	>50	−4.971	−62.93
4	**7e**	25.89	15.61	−4.400	−58.99
5	**7f**	13.97	18.92	−6.174	−59.66
6	**7g**	>50	>50	−6.237	−53.80
7	**7h**	24.08	20.66	−7.769	−58.71
8	**7i**	26.58	29.18	−3.597	−52.57
9	**7j**	>50	>50	−5.719	−52.88
10	**7k**	>50	>50	−5.797	−53.68
11	**7l**	>50	>50	−6.149	−53.49
12	**7m**	13.25	18.37	−6.008	−65.81
13	**BG-45**	31.77	34.33	–	–
14	**Reference ligand**	–	–	−8.510	−71.22

Table 2
ADMET properties of **7a**–**m**.

S. No.	Entry	Donor HB	QlogPo/w	QlogS	% Human oral absorption	Rule of five
1	**7a**	1.5	3.997	−6.172	100	0
2	**7b**	0	6.524	−8.812	100	1
3	**7c**	0	5.717	−7.780	100	1
4	**7d**	0	6.706	−6.794	100	1
5	**7e**	0	5.281	−6.258	100	1
6	**7f**	1	4.468	−5.740	100	0
7	**7g**	0	4.917	−6.504	100	0
8	**7h**	0	4.455	−5.5855	100	0
9	**7i**	0	5.570	−6.315	100	1
10	**7j**	0	5.312	−6.018	100	1
11	**7k**	0	4.969	−5.720	100	1
12	**7l**	1	2.459	−4.331	88.78	0
13	**7m**	0	3.433	−5.865	100	0
14	**BG-45**	–	–	–	–	–
15	**Reference ligand**	–	–	−8.510	−71.22	

ganic layer was distilled off and isolated from diethyl ether (C_2H_5-O-C_2H_5) (50.0 mL), to give alcohol **3** as cream colored solid 5.4 g (75%).

2.1.3. Synthetic procedure for compound 5

To the stirred solution of dichloromethane (CH_2Cl_2) (25.0 mL), dimethyl formamide (DMF) (25.0 mL) and (2-chloro-7-cyclopentyl-7H-pyrrolo[2,3-*d*]pyrimidin-6-yl)methanol **(3)** (5.0 g, 0.019 mol), triethylamine (Et_3N) (6.0 g, 0.059 mol) was charged with methanesulfonyl chloride (CH_3SO_3Cl) (4.5 g, 0.039 mol) at 0–5 °C and stirred for 2.0 h and distilled off dichloromethane (CH_2Cl_2) under vacuum at 25–35 °C. Reaction mass was diluted with dimethyl formamide (DMF) (25.0 mL), charged with Sodium azide (NaN_3) (1.29 g, 0.039 mol) and heated the reaction mass to 75–80 °C with stirring for 3.0 h. Reaction completion was monitored by TLC (solvent is missing) and quenched with chilled water (250.0 mL). Product was extracted in with dichloromethane (CH_2Cl_2) (200.0 mL) and separated the layers. The separated organic layer washed DM water (100.0 mL) and dried over anhydrous sodium sulfate (Na_2SO_4). The obtained organic layer was distilled off and iso-

Table 3
Non-cytotoxicity of 7a–m against normal cell lines.

Entry	7a	7c	7e	7f	7h	7i	7m
IC_{50} Value (μM)	120.1	159.8	145.9	106.0	110.2	147.7	152.9

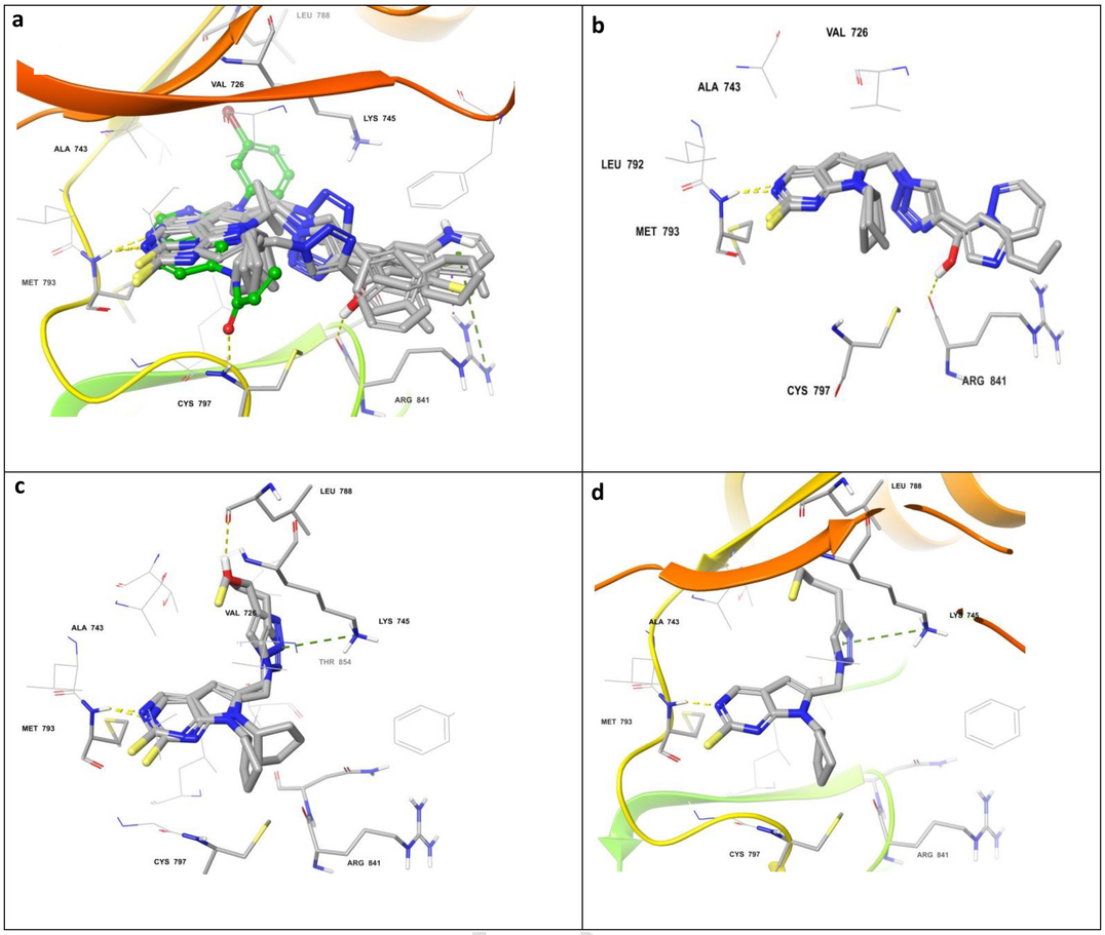


Fig. 5. a Overlap of **7g**, **7a**, **7f**, **7c**, **7k**, **7j**, **7m** and **7b** (macro model thick sticks) with 34-JAB (green thick sticks); 5b and c. Overlap of **7h** and **7l** and **5d**, **7h** at EGFR tyrosine kinase active site. (Yellow dotted line = hbond, green dotted line π-cation and pink dotted line indicates salt bridge interactions). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

lated from diethyl ether (C_2H_5-O-C_2H_5) (60.0 mL), to give azide **5** as yellow colored solid 3.8 g (75%).

2.1.4. Synthesis of compound 7a–m

To a mixture of CuI (4.12 mg, 0.02 mmol, 0.02 equiv), DIPEA (5.6 mg, 0.04 mmol, 0.04 equiv), and HOAc (2.6 mg, 0.04 mmol, 0.04 equiv) and 6-(azidomethyl)-2-chloro-7-cyclopentyl-7H-pyrrolo[2,3-*d*]pyrimidine **5** (300 mg, 1 mmol) in dichloromethane (6.0 mL) was added 3-ethynylaniline **6a** (127 mg, 1 mmol) at room temperature. The resultant mixture was stirred until the absence of acetylene (approximately 24–36 h) and distilled off the solvent completely to get the residual mass. Finally, crude material was purified by a column chromatography [silica gel, 1:1 ratio of EtOAc and Hexane] to give compound **7a** as an off white solid 320 mg (75%). Following the same procedure as depicted for **7a,** the other triazole derivatives **7b–m** were prepared from the corresponding terminal alkynes **6b–m.**

2.2. Biological screening

2.2.1. MTT assay protocol for testing 7a–m

Anti-cancer activity of novel compounds was determined with the help of MTT assay (Van Meerloo et al., 2011). In MTT assay, murine melanoma cell line (B16F10) and breast cancer cell line (MCF-7) were used to screen the compounds. Both the cell lines were cultured in complete Dulbecco's Modified Eagle Medium (DMEM), supplemented with 1% antibiotic and 10% fetal bovine serum. All reagents were obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. The culturing cells were maintained in the incubator with humidified atmosphere and 5% CO_2 at 37 °C. A count of 10,000 cells per well has been seeded on sterile 96 well plates. After overnight incubation, two different doses (100 μM and 10 μM) of compounds were used to treat the cells for 48 h. The medium containing the drug solution had been taken out from the wells after the drug treatment and followed by addition of 50 μL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (5 mg/mL) in phenol red free medium into the well and allowed to incubate for another 4 h. Then an equal volume of DMSO was added in to each well to dissolve the so formed purple colour formazan crystals. The absorbance was measured at 570 nm and 650 nm by using Spectramax (Molecular Devices, USA) after a gentle shaking of the well plates. Percentage of cell viability was calculated considering the DMSO treated wells have 100% viable cells. Some of the compounds in the initial screen were found to possess good anti-cancer activity. Compounds denoted as, 7a, 7c, 7e, 7f, 7h, 7i and 7m were found to show promising activity. These compounds were further tested for determination of IC_{50} value with a wide range of concentrations. The similar procedure was followed as described before. There ten different concentrations such as 200 μM, 100 μM, 50 μM, 25 μM, 12.5 μM, 6.25 μM, 3.125 μM, 1.562 μM, 0.781 μM and 0.390 μM were used to test the selected compounds. Another batch of cells had been used in the repeat of the experiment.

2.2.2. Molecular docking protocol

The X-ray crystallographic 3D structure of EGFR tyrosine kinase (PDB: 2J5F) was obtained from protein data bank (Blair et al., 2007).

The monomeric protein was then prepared by the Protein Preparation Wizard and its energy was minimized by Epik. The reference ligand *i.e.*, 34-JAB was employed for receptor grid generation. All the ligands were sketched in chemdraw and converted in to desired forms by Meastro 11.6 and prepared using LigPrep. The ligand docking was carried out in extra precision docking mode of the Glide and results were analyzed.

3. Results and discussion

3.1. Synthesis of 7a to m

The designed synthetic route for the synthesis of targeted compounds **7a–m** is shown in Scheme 1. The first step of the synthesis involves the esterification of commercially available 2-chloro-7-cyclopentyl-7H-pyrrolo[2,3-*d*]pyrimidine-6-carboxylic acid **1** in the presence of methanol and catalytic amount of H_2SO_4 (Schreiber, 2000; Kuruvilla et al., 2002; Wipf et al., 2004; Taylor et al., 2004), to yield methyl 2-chloro-7-cyclopentyl-7H-pyrrolo[2,3-*d*]pyrimidine-6-carboxylate **2**. In the second step, reduction of **2** with $NaBH_4$ in THF-methanol (Hughes et al., 2011; Anderson, 2003; Prachayasittikul et al., 2014) yielded the alcohol **3**. Further, alcohol **3** was converted to azide **5** by treating with NaN_3 in DMF (Rogozinski, 1964) *via in situ* conversion of hydroxy group in to reactive mesylated intermediate **4.** The mesylated intermediate **4** was generated using methanesulfonyl chloride in DCM, TEA and catalytic DMF (Bianco et al., 1988). Finally, the targeted compounds, hybrid molecules of pyridine-pyrrole appended substituted triazoles **7a–m** were synthesized *via* 1, 3-dipolar cycloaddition of azide **5** and terminal alkynes **6a–m** in the presence of CuI, DIPEA and catalytic amount of CH_3COOH (Conrow and Dean, 2008). All these newly synthesized compounds were purified by column chromatography and characterized by Mass, ^1H NMR and ^{13}C NMR.

Theoretically in 1,3-dipolar cycloaddition, azide and terminal alkyne produce a mixture of 1,4- and 1,5-disubstituted triazoles. Several attempts were made to control the regioselectivity. The discovery of the copper(I) catalyzed reaction in 2002, exclusively yields the 1,4-disubstituted 1,2,3-trizoles (Oral et al., 2009; Shao et al., 2011). In the absence of Cu(I) catalyst, the original 1,3-dipolar Huisgen cycloaddition of azides and terminal alkynes are not regioselective and usually slow (Creary et al., 2012). The use of catalytic amount of copper (I), which binds to the terminal alkynes, leads to the regioselective azide-alkyne cycloaddition.

The schematic representation for the formation of 1,2,3-triazoles **7a–m** is shown in Scheme 2. Based on the experimental evidence, as like Sonogashria reaction, the Cu(I) metal is inserted readily into terminal alkynes and involves in the following sequential mechanistic steps. In the first step, Cu(I) is involved in the complexation of alkyne **6a** to give **6b**. Further the deprotonation of the terminal hydrogen leads to the formation of a complex *Cu*-acetylide **6c** (Rostovtsev et al., 2002; Tornøe et al., 2002). In the next step, proximal N(l) displaces one of the ligands from the second *Cu*-acetylide complex to give intermediate **6d** which "activates" the azide for nucleophilic attack C(4). In the next step due to proximity and electronic factors, N(3) can now easily attack C(4) of the alkyne, leading to a metallocycle **(6e)**. Finally metallocycle then contracts when the lone pair of electrons of N(l) attacks at C(5) to form 1,2,3-triazole **(6f)**. Protonation by the base (H_2O) releases the Cu(I) catalyst to form the 1,2,3-triazole **7a**, the catalyst cycle continues with Cu(I) (Cu_2L_2) complexing with a terminal alkyne.

To demonstrate the structure elucidation of the pyrimidine-pyrrole appended substituted triazole derivatives **7a–m**, we selected compound **7a** which was obtained by the reaction of equimolar quantities of azide **5** and terminal alkyne **6a** in the presence of CuI, DIPEA and catalytic amount of CH_3COOH for 3 h. Its positive quasi molecular ion peak was observed at *m/z* 394.2 (M + H), compatible with the molecular formulae $C_{20}H_{20}ClN_7$. In ^1H NMR of **7a**, the newly formed triazole ring H-4 appeared as singlet at δ 8.43. In ^{13}C NMR of **7a,** triazole ring carbons appeared at δ 147.3 (C-5) and130.8 (C-4). Absence of azide (N═N═N) stretching value in IR at 2117 cm^{-1} also confirmed the product formation.

3.1.1. Methyl 2-chloro-7-cyclopentyl-7H-pyrrolo[2,3-d]pyrimidine-6-carboxylate (2)

IR (KBr, cm^{-1}): 1725.45 (C═O), 1135.11 (C—O—C), 750.87 (C—Cl); ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 9.11 (s, 1H, phenyl-H), 7.43 (s, 1H, pyrrole-H), 5.76–5.64 (m, 1H, cyclopentyl-H), 3.90 (s, 3H, -CH_3), 2.32–2.23 (m, 2H, cyclopentyl-H), 2.04–2.02 (m, 4H, cyclopentyl-H), 1.69–1.66 (m, 2H, cyclopentyl-H);^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 160.84, 155.03, 154.05, 152.27, 130.09, 116.15, 108.65, 56.33, 52.50, 30.41, 24.62. MS (ESI) *m/z* 280.20 [M + H], 282.15 [M + H] $^{+2}$.

3.1.2. (2-chloro-7-cyclopentyl-7H-pyrrolo[2,3-d]pyrimidin-6-yl)methanol (3)

IR (KBr, cm^{-1}): 3352.75 (O—H), 770.85 (C—Cl); ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 8.83 (s, 1H, phenyl-H), 6.56 (s, 1H, pyrrole-H), 5.54–5.52 (t, 1H, —OH), 4.90–4.84 (m, 1H, cyclopentyl-H), 4.69–4.68 (d, 2H, -CH_2), 2.28–2.27 (m, 2H, cyclopentyl-H), 2.25–1.97 (m, 4H, cyclopentyl-H), 1.68–1.67 (m, 2H, cyclopentyl-H); ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 151.86, 151.03, 155.54, 144.19, 117.73, 98.10, 55.99, 55.84, 30.24, 24.48. MS (ESI) *m/z* 252.23 [M + H], 254.24 [M + H] $^{+2}$.

3.1.3. 6-(azidomethyl)-2-chloro-7-cyclopentyl-7H-pyrrolo[2,3-d]pyrimidine (5)

IR (KBr, cm^{-1}): 2117.67 (—N═N═N), 1374.72 (C—N), 775.71 (C—Cl); ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 8.93 (s, 1H, phenyl-H), 6.78 (s, 1H, pyrrole-H), 4.83–4.77 (m, 3H, cyclopentyl-H and -CH_2), 2.31–2.26 (d, 2H, cyclopentyl-H), 2.07–2.00 (m, 4H, cyclopentyl-H), 1.70–1.66 (m, 2H, cyclopentyl-H); ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 151.83, 151.78, 151.40, 137.47, 117.36, 100.77, 56.16, 45.96, 30.26, 24.49. MS (ESI) m/z 277.18 [M + H], 279.13[M + H] $^{+2}$.

3.1.4. 3-(1-((2-chloro-7-cyclopentyl-7H-pyrrolo[2,3-d]pyrimidin-6-yl)methyl)-1H-1,2,3-triazol-4-yl)aniline (7a)

Yield:74%; Brown colored solid, M.Pt.: 179 °C; IR (KBr, cm^{-1}): 3140.38 (—N═N═N), 1380.30 (C—N), 790.69 (C—Cl); ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 8.93 (s, 1H, phenyl-H), 8.43 (s, 1H, tiazole-H), 7.11–7.04 (m, 2H, phenyl-H), 6.95–6.93 (m, 1H, phenyl-H), 6.71 (s, 1H, pyrrole-H), 6.54–6.52 (d, 1H, phenyl-H), 6.03 (s, 2H, —CH_2), 4.88–4.84 (p, 1H, cyclopentyl-H), 2.22–2.18 (m, 2H, cyclopentyl-H), 1.99–1.91 (m, 4H, cyclopentyl-H), 1.62–1.59 (m, 2H, cyclopentyl-H);^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 151.73, 151.62, 148.60, 147.32, 137.45, 130.87, 129.34, 121.17, 117.57, 113.91, 113.35, 110.70, 101.04, 56.26, 45.69, 30.11, 24.52. MS (ESI) *m/z* 394.20 [M + H], 396.20 [M + H] $^{+2}$.

3.1.5. 2-chloro-7-cyclopentyl-6-((4-(phenanthren-9-yl)-1H-1,2,3-triazol-1-yl)methyl)-7H-pyrrolo[2,3-d]pyrimidine (7b)

Yield, 65%; Yellow colored solid, M.Pt.: 208 °C; IR (KBr, cm^{-1}): 3124.6 (—N═N═N), 1376.70 (C—N), 796.69 (C—Cl);^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 8.78 (s, 1H, pyrimidine-H), 8.32 (s, 1H, tiazole-H), 8.84–8.82 (m, 2H, phenyl-H), 8.78–8.75 (m, 3H, phenyl-H), 7.11–7.04 (m, 3H, phenyl-H), 6.95–6.93 (m, 1H, phenyl-H), 6.78 (s, 1H, pyrrole-H), 6.21 (s, 2H, —CH_2), 4.82–4.80 (p, 1H, cyclopentyl-H), 2.19–2.17 (m, 2H, cyclopentyl-H), 1.95–1.92 (m, 4H, cyclopentyl-H), 1.59–1.57 (m, 2H, cyclopentyl-H);^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 150.62, 149.78, 147.60,

146.29, 134.45, 130.17, 129.34, 128.31, 126.65, 122.25, 121.27, 119.42, 113.98, 113.35, 109.69, 101.02, 55.26, 53.72, 30.12, 24.45. MS (ESI) *m/z* 479.12 [M + H], 481.24 [M + H] $^{+2}$.

3.1.6. 2-chloro-7-cyclopentyl-6-((4-(3,4-dichlorophenyl)-1H-1,2,3-triazol-1-yl)methyl)-7H-pyrrolo[2,3-d]pyrimidine (7c)

Yield, 77%; Cream colored solid, M.Pt.: 180 °C; IR (KBr, cm^{-1}): 3145.91 (—N═N═N), 1379.50 (C—N), 747.77 (C—Cl); ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 8.95 (s, 1H, pyrimidine-H), 8.72 (s, 1H, phenyl-H), 8.11 (s, 1H, triazole-H), 7.89–7.86 (dd, 1H, phenyl-H), 7.71–6.69 (d, 1H, phenyl-H), 6.76 (s, 1H, pyrrole-H), 6.09 (s, 2H, —CH_2),4.85–4.80 (p, 1H, cyclopentyl-H), 2.20–2.16 (m, 2H, cyclopentyl-H), 2.01–1.97 (m, 2H, cyclopentyl-H), 1.84–1.77 (m, 2H, cyclopentyl-H); 1.65–1.57 (m, 2H, cyclopentyl-H);^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 151.71, 144.48, 136.93, 131.72, 131.13, 130.26, 126.79, 125.16, 122.60, 117.57, 101.38, 56.27, 45.89, 30.14, 24.53. MS (ESI) *m/z* 447.0 [M + H], 449.0 [M + H] $^{+2}$.

3.1.7. 6-((4-([1,1′-biphenyl]-4-yl)-1H-1,2,3-triazol-1-yl)methyl)-2-chloro-7-cyclopentyl-7H-pyrrolo[2,3-d]pyrimidine (7d)

Yield, 79%; Yellow colored solid, M.Pt.: 245 °C; IR (KBr, cm^{-1}): 3107.90 (—N═N═N), 1380.27 (C—N), 766.68 (C—Cl); ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 8.95 (s, 1H, pyrimidine-H), 8.65 (s, 1H, triazole-H), 7.96–7.94 (d, 2H, phenyl-H), 7.76–7.70 (m, 4H, phenyl-H), 7.49–7.45 (t, 2H, phenyl-H), 7.39–7.35 (t, 1H, phenyl-H), 6.75 (s, 1H, pyrrole-H), 6.08 (s, 2H, —CH_2),4.90–4.82 (m, 1H, cyclopentyl-H), 2.23–2.20 (m, 2H, cyclopentyl-H), 1.99–1.81 (m, 2H, cyclopentyl-H), 1.63–1.60 (m, 2H, cyclopentyl-H); 1.27–1.23 (m, 2H, cyclopentyl-H);^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 151.69, 146.32, 139.51, 137.29, 129.50, 128.94, 127.54, 127.11, 126.50, 125.73, 121.72, 117.61, 101.18, 56.28, 45.81, 30.13, 24.54. MS (ESI) *m/z* 455.2 [M + H], 457.2 [M + H] $^{+2}$.

3.1.8. 2-chloro-7-cyclopentyl-6-((4-(4-methoxy-2-methylphenyl)-1H-1,2,3-triazol-1-yl)methyl)-7H-pyrrolo[2,3-d]pyrimidine (7e)

Yield, 82%; Yellow colored solid, M.Pt.: 124 °C; IR (KBr, cm^{-1}): 3117.46 (—N═N═N), 1378.42 (C—N), 760.52 (C—Cl); ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 8.92 (s, 1H, pyrimidine-H), 8.35 (s, 1H, triazole-H), 7.63–7.61 (d, 1H, phenyl-H), 6.88–6.83 (m, 2H, phenyl-H), 6.69 (s, 1H, pyrrole-H), 6.04 (s, 2H, —CH_2), 4.94–4.86 (m, 1H, cyclopentyl-H), 3.77 (s, 3H, Ar-OCH_3), 2.38 (s, 3H, Ar-CH_3), 2.24–2.18 (m, 2H, cyclopentyl-H), 2.01–1.91 (m, 2H, cyclopentyl-H), 1.84–1.82 (m, 2H, cyclopentyl-H), 1.66–1.61 (m, 2H, cyclopentyl-H);^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 158.82, 151.72, 151.58, 145.86, 137.65, 136.56, 129.58, 122.83, 122.35, 117.56, 115.99, 111.54, 100.85, 56.27, 55.20, 45.64, 30.09, 24.50, 21.17. MS (ESI) *m/z* 423.12 [M + H], 425.13 [M + H] $^{+2}$.

3.1.9. 1-(1-((2-chloro-7-cyclopentyl-7H-pyrrolo[2,3-d]pyrimidin-6-yl)methyl)-1H-1,2,3-triazol-4-yl)hexan-1-ol (7f)

Yield, 81%; Pale brown colored solid, M.Pt.: 125 °C; IR (KBr, cm^{-1}): 3141.37 (—N═N═N), 1373.68 (C—N), 765.02 (C—Cl); ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 8.92 (s, 1H, pyrimidine-H),7.92 (s, 1H, triazole-H), 6.69 (s, 1H, pyrrole-H), 5.96 (s, 2H, —CH_2), 5.18 (s, 1H, —OH), 4.86–4.82 (t, 1H, hexyl-H), 4.63(m, 1H, cyclopentyl-H), 2.18–1.59(m, 11H, cyclopentyl &hexyl-H), 1.22(m, 8H, hexyl-H), ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm:152.33, 151.62, 137.72, 127.83, 121.85, 117.51, 100.92, 65.47, 56.22, 45.37, 40.12, 38.87, 37.23, 31.13, 30.00, 24.46, 22.04, 13.81. MS (ESI) *m/z* 403.17 [M + H], 405.12 [M + H] $^{+2}$.

3.1.10. 2-chloro-7-cyclopentyl-6-((4-(p-tolyl)-1H-1,2,3-triazol-1-yl)methyl)-7H-pyrrolo[2,3-d]pyrimidine(7g)

Yield, 84%; Cream colored solid, M.Pt.: 192 °C; IR (KBr, cm^{-1}): 3135.68 (—N═N═N), 1375.76 (C—N), 751.01(C—Cl); ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 8.93 (s, 1H, pyrimidine-H),8.53 (s, 1H, triazole-H), 7.74–7.72 (d, 2H, phenyl-H), 7.24–7.22 (d, 2H, phenyl-H), 6.72 (s, 1H, pyrrole-H), 6.04 (s, 2H, -CH_2), 4.88–4.83 (p, 1H, cyclopentyl-H), 2.31 (s, 3H, phenyl-CH_3), 2.25–2.15 (m, 2H, cyclopentyl), 2.01–1.93 (m, 2H, cyclopentyl), 1.85–1.78 (m, 2H, cyclopentyl-H), 1.65–1.58 (m, 2H, cyclopentyl-H);^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm:151.73, 151.63, 146.73, 137.35, 129.38, 127.64, 125.11, 121.16, 117.58, 101.09, 56.26, 45.74, 30.11, 24.52, 20.77. MS (ESI) *m/z* 393.2 [M + H], 395.2 [M + H] $^{+2}$.

3.1.11. 2-chloro-6-((4-(3-chloropropyl)-1H-1,2,3-triazol-1-yl)methyl)-7-cyclopentyl-7H-pyrrolo[2,3-d]pyrimidine (7h)

Yield, 78%; Pale yellow colored solid, M.Pt.: 112 °C; IR (KBr, cm^{-1}): 3134.45 (—N═N═N), 1370.93 (C—N), 756.41(C—Cl); ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 8.92 (s, 1H, pyrimidine-H), 7.94 (s, 1H, triazole-H), 6.66 (s, 1H, pyrrole-H), 5.95 (s, 2H, —CH_2), 4.86–4.77 (q, 1H, cyclopentyl-H), 3.67–3.64 (t, 2H, propyl-H), 2.77–2.74 (t, 2H, propyl-H), 2.21–2.16 (m, 2H, cyclopentyl-H), 2.05–1.59 (m, 4H, cyclopentyl &propyl-H), 1.80–1.77 (m, 2H, propyl-H);1.62 (m, 2H, cyclopentyl-H);^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm:151.68, 151.57, 145.96, 137.64, 122.42, 117.52, 100.82, 56.21, 45.45, 44.54, 31.70, 30.03, 24.47, 22.21. MS (ESI) *m/z* 379.06 [M + H], 381.08 [M + H] $^{+2}$.

3.1.12. 2-chloro-7-cyclopentyl-6-((4-heptyl-1H-1,2,3-triazol-1-yl)methyl)-7H-pyrrolo[2,3-d]pyrimidine (7i)

Yield, 79%; Pale brown colored solid, M.Pt.: 105 °C; IR (KBr, cm^{-1}): 3135.21 (—N═N═N), 1370.66 (C—N), 755.25 (C—Cl); ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 8.92 (s, 1H, pyrimidine-H), 7.86 (s, 1H, triazole-H), 6.67 (s, 1H, pyrrole-H), 5.94 (s, 2H, —CH_2), 4.85–4.77 (q, 1H, cyclopentyl-H), 2.62–2.58 (t, 2H, heptyl-H), 2.20–2.15 (m, 2H, cyclopentyl-H), 1.98–1.95 (m, 2H, cyclopentyl-H), 1.78–1.76 (m, 2H, cyclopentyl-H), 1.61–1.54 (m, 4H, cyclopentyl & heptyl-H), 1.25–1.20 (m, 8H, heptyl-H), 0.84–0.81 (m, 3H, heptyl-H);^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm:151.68, 151.58, 147.33, 137.71, 122.04, 117.52, 100.84, 56.19, 45.38, 31.15, 30.00, 28.87, 28.33, 24.82, 24.45, 21.97, 13.85. MS (ESI) *m/z* 400.99 [M + H], 402.91 [M + H] $^{+2}$.

3.1.13. 2-chloro-7-cyclopentyl-6-((4-hexyl-1H-1,2,3-triazol-1-yl)methyl)-7H-pyrrolo[2,3-d]pyrimidine (7j)

Yield 79%; Cream colored solid, M.Pt.: 114 °C; IR (KBr, cm^{-1}): 3136.99 (—N═N═N), 1371.03 (C—N), 753.96 (C—Cl); ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 8.92 (s, 1H, pyrimidine-H), 7.87 (s, 1H, triazole-H), 6.67 (s, 1H, pyrrole-H), 5.95 (s, 2H, —CH_2), 4.86–4.78 (q, 1H, cyclopentyl-H), 2.62–2.58 (t, 2H, hexyl-H), 2.21–2.16(m, 2H, cyclopentyl-H), 2.00–1.92(m, 2H, cyclopentyl-H), 1.78–1.76 (m, 2H, cyclopentyl-H), 1.61–1.54 (m, 4H, hexyl-H), 1.24(m, 6H, hexyl-H), 0.83–0.81(m, 3H, hexyl-H); ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 151.9, 151.55, 147.34, 137.71, 122.04, 117.53, 100.83, 56.21, 44.40, 30.94, 30.01, 28.87, 28.07, 24.86, 24.47, 21.98, 13.81. MS (ESI) *m/z* 387.03 [M + H], 388.95 [M + H] $^{+2}$.

3.1.14. 2-chloro-7-cyclopentyl-6-((4-pentyl-1H-1,2,3-triazol-1-yl)methyl)-7H-pyrrolo[2,3-d]pyrimidine (7k)

Yield 77%; Cream colored solid, M.Pt.: 125 °C; IR (KBr, cm^{-1}): 3135.05 (—N═N═N), 1370.86 (C—N), 756.41 (C—Cl); ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 8.91 (s, 1H, pyrimi-

dine-H), 7.86 (s, 1H, triazole-H), 6.66 (s, 1H, pyrrole-H), 5.93 (s, 2H, -CH_2), 4.83–4.78 (q, 1H, cyclopentyl-H), 2.61–2.57 (t, 2H, pentyl-H), 2.18–2.15(m, 2H, cyclopentyl-H), 1.96 (m, 2H, cyclopentyl-H), 1.77–1.75(m, 2H, cyclopentyl-H), 1.59–1.54 (m, 4H, cyclopentyl-H& pentyl-H), 1.26–1.22(m, 4H, pentyl-H), 0.85–0.81(t, 3H, pentyl-H); ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 151.69, 151.59, 147.34, 137.71, 122.05, 117.53, 100.82, 56.20, 45.39, 30.64, 30.00, 28.58, 24.80, 24.45, 21.78, 13.81. MS (ESI) *m/z* 372.99 [M + H], 374.98 [M + H] $^{+2}$.

3.1.15. (1-((2-chloro-7-cyclopentyl-7H-pyrrolo[2,3-d]pyrimidin-6-yl)methyl)-1H-1,2,3-triazol-4-yl)methanol (7l)

Yield 74%; Brown colored solid, M.Pt.: 151 °C;IR (KBr, cm^{-1}): 3148.18 (—N═N═N), 1375.20 (C—N), 772.31 (C—Cl); ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 8.92 (s, 1H, pyrimidine-H), 8.02 (s, 1H, triazole-H), 6.65 (s, 1H, pyrrole-H), 5.98 (s, 2H, —CH_2), 5.19 (t, 1H, —OH), 4.91–4.86 (q, 1H, cyclopentyl-H), 4.53–4.52 (d, 2H, hydroxyl-CH_2), 2.20–2.17 (m, 2H, cyclopentyl-H), 1.99–1.82(m, 4H, cyclopentyl-H), 1.64–1.61 (m, 2H, cyclopentyl-H); ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 151.59, 151.53, 137.71, 123.12, 117.43, 100.68, 56.17, 54.90, 53.53, 45.36, 30.02, 24.44. MS (ESI) m/z 332.94 [M + H], 335.02 [M + H] $^{+2}$.

3.1.16. 2-chloro-7-cyclopentyl-6-((4-(imidazo[1,2-b]pyridazin-3-yl)-1H-1,2,3-triazol-1-yl)methyl)-7H-pyrrolo[2,3-d]pyrimidine (7m)

Yield 76%; Yellow colored solid, M.Pt.: 202 °C IR (KBr, cm^{-1}): 3163.97 (—N═N═N), 1377.39 (C—N), 749.99 (C—Cl); ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 8.92 (s, 1H, pyridazine-H), 8.85 (s, 1H, pyrimidine-H), 8.74 (s, 1H, traizole-H), 8.36 (bs, 2H, pyridazine-H), 7.39 (s, 1H, pyrazole-H), 6.79–6.73 (s, 1H, pyrrole-H), 6.18 (s, 2H, —CH_2), 5.02–4.94 (q, 1H, cyclopentyl-H), 2.25–2.20 (m, 2H, cyclopentyl-H), 1.98–1.88 (m, 4H, cyclopentyl-H), 1.65–1.62(m, 2H, cyclopentyl-H); ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 159.75, 153.26, 152.23, 149.86, 142.23, 136.35, 132.12, 131.52, 130.95, 126.56, 123.58, 124.21, 120.32, 118.95, 113.25, 65.78, 36.54, 23.91. MS (ESI) m/z 420.02 [M + H], 422.02 [M + H] $^{+2}$.

3.2. Biological screening

3.2.1. Anti-cancer activity of 7a–m

The newly synthesized pyrrolopyrimidine based triazoles (**7a–m**) were screened for their ant-cancer activity using melanoma (B16F10) and breast cancer (MCF-7) cell lines employing MTT assay. The synthesized library consists of different functional groups such as aliphatic chains with increasing carbons, alkyl halides, alcohols, aromatic rings with *mono* and *di* substitutions, aryl, biaryl, imidazopyrimidine and anthracenyl. The compound with imidazopyrimidine (**7m**, IC_{50} = 13.25 and 18.37 μM) and alcohol groups (**7f**, IC_{50} = 13.97 and 18.92 μM) exhibited highest anti-cancer activity among the tested ones, against both cell lines. The *di*-chloro substituted compound (**7c**, IC_{50} = 19.65 and 23.69 μM) displayed second maximum activity against two cell lines. The 4-methoxy, 2-methyl derivative exhibited its maximum activity against MCF-7 (IC_{50} = 15.61 μM) when compared to B16F10 cells (IC_{50} = 25.89 μM). The alkyl halide (**7h**) exerted the best anti-cancer efficacy towards MCF-7 (IC_{50} = 20.66 μM) than B16F10 cells (IC_{50} = 20.66 μM). The aniline derivative (**7a**) displayed similar anti-cancer activity (IC_{50} = 26.20 and 27.89 μM) against both tested cell lines. The alkylated triazole (**7i**, IC_{50} = 26.58 and 29.18 μM) displayed the least anti-cancer effect like **7a** in both tested cells. Other tested compounds **7d**, **7j**, **7k**, **7l** (biphenyl, hexyl, pentyl and alcohol substituted) were prone to have less activity (>50 μM) in tested concentrations. The compounds **7c** and **7f** were proven to have better activity against melanoma cells and **7e** against breast cancer cell lines. The preliminary anti-cancer activity [Figs. 2 and 3] and the IC_{50} data were delineated in Fig. 4 A and B, and in Table 1 along with docking scores and binding free energies. The drug like parameters of **7a**–**m** were also predicted using Qikprop and provided in the Table 2. To know whether the current compounds are meeting the drug like properties or not, they have been subjected for QikProp studies and most of the compounds were found to follow the Lipinski rule.

3.2.2. Structure and activity relationship

Nature of substitutent on triazole dictates the activity of the synthesized pyrimidine-pyrrole appended substituted triazole derivatives. Higher activity was observed with polar substituents (pyradizine, hexanol dichlorobenzene, chloro propane, methoxy methyl benzene) and moderate activity with unsubsituted aromatic/higher alkane (aniline and heptane). Least activity was observed with substitution of polynuclear/alkly substituted aromatic hydrocarbons (phenanthrene, phenylbenzene and methylbenzene) and lower alkanes (hexane and pentane).

3.2.3. Cytotoxicity of 7a–m against normal cell lines

The promising compounds from triazole series were screened further to find out their cytotoxic IC_{50} values with non-cancerous normal cell lines. 7a, 7c, 7e, 7f, 7h, 7i and 7m were evaluated in ten different doses on human embryonic kidney cells (HEK-293) for 48 h and cell viability was measured using MTT assay (Table-3). The selected synthesized compounds are less cytotoxic towards the normal cell lines (human embryonic kidney cells, HEK-293). Hence, they can be utilized as anticancer agents for the treatment of cancer cells (MCF-7 and B16F10) (Table 3).

3.3. Molecular docking analysis

The molecular docking studies indicating EGFR tyrosinse (PDB: 2J5F) as putative target in the current series of compounds (**7a** to **m**) was carried out. The binding site of 34-JAB (reference ligand: N-[4-(3-bromophenylamino) quinazolin-6-yl] acrylamide) is well defined by the hydrophobic cavity lined with active residues like Cys797, Met793, Thr790, Thr854, Asp855, Arg841, Lys745 and Leu788. The EGFR tyrosine kinase with several anti-cancer pyrimidine and its allied derivatives has been reported as effective druggable target which is vital for cancer cell survival. The extra precision docking mode of the Glide was employed for the current docking protocol. The two typical binding modes of the current ligands have been observed within the active cavity of the EGFR tyrosine kinase. The preliminary observation from binding nature of ligands **7a**–**m,** the pyrrolopyrimidine nucleus was occupied as similar to the quinazoline ring of 34-JAB when overlapped in the active site and established the crucial hbond with the Met793 (EJMC, 155, 316–336, 2018). The ligands **7g**, **7a**, **7f**, **7c**, **7k**, **7j**, **7m** and **7b** exhibited comparable binding orientation within the active site of the EGFR tyrosine kinase (Fig. 5a). The most active ligands **7f** and **7m** are also depicted the similar binding orientation in the active site (Fig. 5b). Among these set of ligands, the *di*-chloro derivative (**7c**) depicted salt bridge interaction with guanidine of Arg841 and alcohol group of **7f** displayed hbond with the backbone amide of the Arg841. The phenanthrenyl ligand (**7b**) contributed hbond with guanidine of Arg841. The other set of ligand with alkyl halide (**7h**) and terminal alcohol (**7l**) displayed different binding alignment when compared with earlier ones. The triazole of **7h** displayed π-cation with Lys745 and alcohol of **7l** exhibited hbond with Leu788. The alignment of pyrrolopyrimidine nucleus of the other ligands (**7d, 7i and 7e**) *i.e.*, biphenyl derivative (**7d**), aliphatic heptyl (**7i**) and methoxy analogs (**7e**) were not found to be similar as mentioned earlier and might be unfavorable at accommodative site.

The docking data clearly demonstrated the orientation of the basic pyrrolopyrimidine nucleus at EGFR tyrosine imperative site and contributing essential hbond with backbone of Cys797. The methylene be-

tween basic core and triazole is highly flexible and that might be a valid reason behind the accommodation of triazoles with aryl ring in other positions within the active site (Figs. 5a–d). The current molecular docking investigation is in relatively close to the anti-cancer efficacy of the **7a** to **m.**

Further, the favorable anti-cancer activity of active compounds with alcohol (**7f**) and heteroclycle (**7m**) might be exerted due to their similar accommodative capability in the active site as reference ligand which made hbond with Met793. Whereas, ligand with bulkier biphenyl (**7d)** and *di*-substituted (**7e)** were found to be occupying in different manner but they do not made any crucial interactions and this finding is approachable with biological screening. The current molecular docking investigation is in relatively close to the anti-cancer efficacy of the **7a** to **m**.

4. Conclusion

In summary, we have successfully achieved two important aspects in this work. First one is the development of an efficient as well as milder method to prepare novel hybrid molecules of pyrimidine-pyrrole appended substituted triazoles **7a–m** in a good yields *via* 1,3-diploar cycloaddition. Second one is, coupling of different pharmacophores, each endowed with diverse biological properties resulted in hybrid molecules with significant antitumor activity. Interestingly, some of the new compounds showed moderate to good activity against tested melanoma and breast cancer cell lines. Higher activity was observed with polar substituents (pyradizine, hexanol, dichlorobenzene, chloro propane, methoxy methyl benzene) on triazole moiety. The observed activity profile suggested that electron withdrawing groups on triazole heterocycle improved the anticancer activity. The binding mode analysis depicted that, the orientation of pyrrolopyrimidine nucleus is similar to the basic ring of the reference ligand and contributed the desired interactions with the active site residues of the EGFR tyrosine kinase. The present strategy behind the synthesis of desired compounds might be fruitful in next of its applications in anti-cancer medicinal chemistry.

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Conflicts of interest

"The authors declare no conflicts of interest".

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