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Synthesis, biological evaluation and docking studies of 1,2,4-oxadiazole linked 5-fluorouracil derivatives as anticancer agents

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Abstract

Background: 1,2,4-oxadiazole derivatives exhibited significant anti-cancer activity when they were evaluated, against human cancer cell lines. They also showed anti-inflammatory, analgesic, diabetic, immunosuppressive, α, β_3 -receptor antagonist, antimicrobial, anti-helminthic, histamine-H3 and antiparasitic properties. A pyrimidine analog, 5-fluoro-uracil is a chemotherapeutic drug used for treating multiple solid malignant tumors. But its application is limited, as it has side effects like low bioavailability and high toxicity. Molecular docking is an exemplary tool, helps in identifying target and designing a drug containing high bio-availability and minimum toxicity.

Results: A set of 1,2,4-oxadiazole linked 5-fluorouracil derivatives (7a–j) were synthesized and their structures were confirmed by ¹HNMR, ¹³CNMR and Mass spectral analysis. Further, these compounds were investigated for their anti-cancer activity towards a panel of four human cancer cell lines such as (MCF-7, MDA MB-231), lung cancer (A549) and prostate cancer (DU-145) by using MTT method. Among them, compounds 7a, 7b, 7c, 7d and 7i demonstrated more promising anticancer activity than standard.

Conclusion: Synthesized derivatives (7a–j) of 1,2,4-oxadiazole linked 5-fluorouracil and investigated for their anticancer activity towards a panel of four human cancer cell lines.

Keywords: 5-Fluorouracil, Ataluren, Pyrimidine, Oxadiazole and anticancer activity

Background

Over the past few decades, heterocyclic rings containing nitrogen atoms have played a significant role in medicinal chemistry. They are considered as key templates for the development of new therapeutic agents [1]. Among all the nitrogenated compounds, pyrimidines are a more privileged class of six-membered heterocyclic organic units. They occupy a unique position in medicinal chemistry due to their wide range of biological applications [2–12]. Pyrimidines exist as an essential component in several

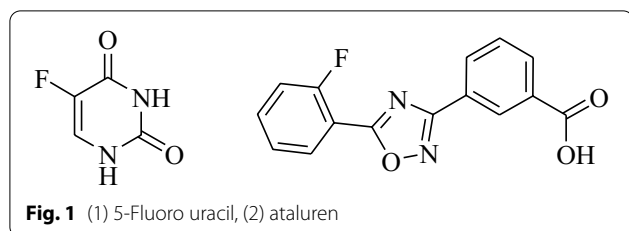
nucleic acids and therapeutic drugs, such as 5-Fluorouracil (1, 5-FU, Fig. 1) [13–16]. The USFDA-approved drug, 5-FU, is one of the most distinguishable chemotherapeutic drugs available. It was first synthesized by Heidelberger and co-workers [17]. It shows antitumor activity by inhibition of thymidylate synthetase enzyme leading to prevention of DNA synthesis [18], and has been used frequently for the treatment of various solid malignant tumors [19–21]. However, it has limited clinical applications because of several side effects, including poor tumor selectivity, toxicity, lower drug-resistance, gastrointestinal toxicity, and adverse effects on central nervous system [22, 23]. Previously, many researchers have developed several 5-FU contained compounds to overcome

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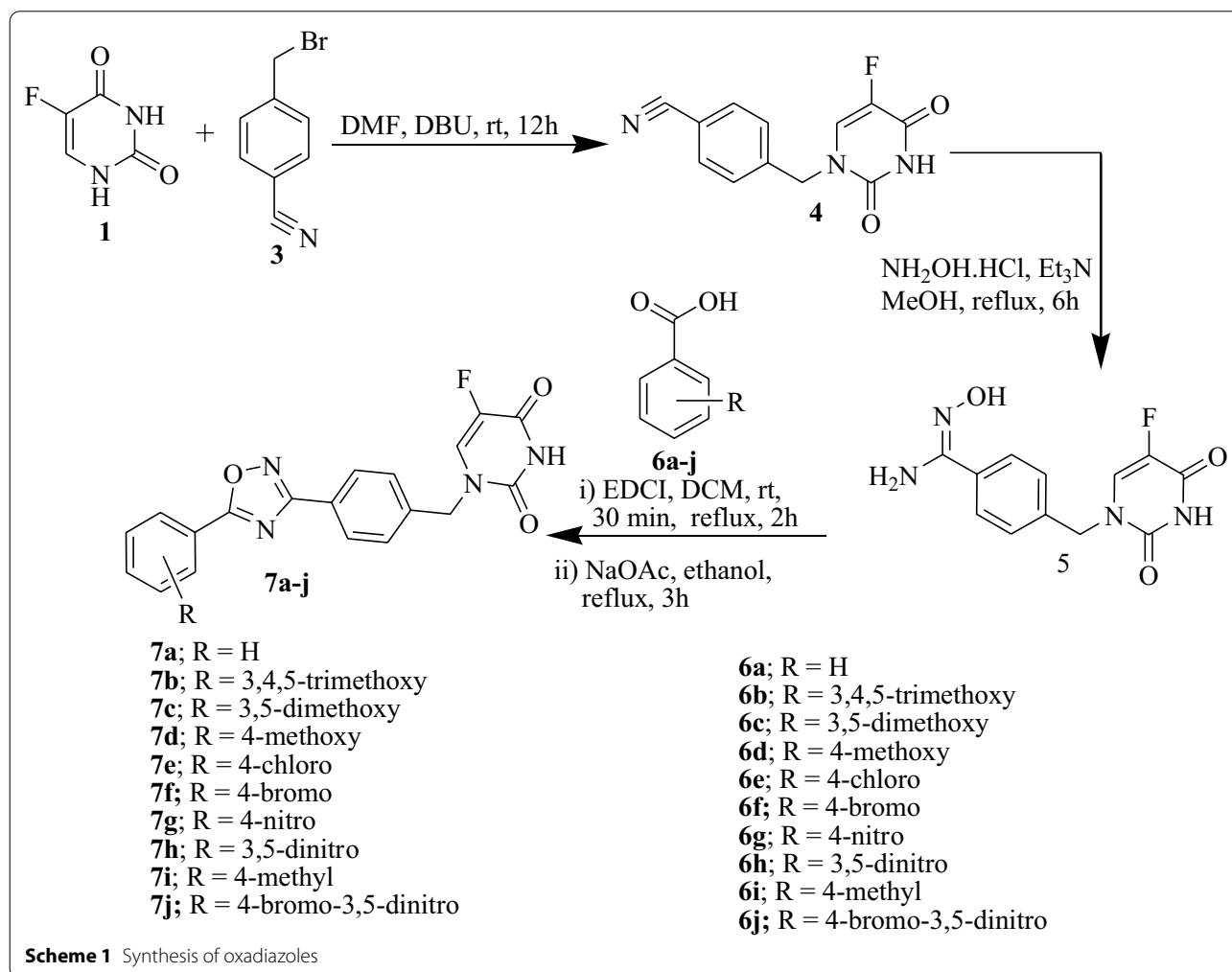
such side effects [24]. On the other hand, oxadiazoles are a unique class of nitrogen and oxygen atoms containing five-membered ring heterocyclic core units [25]. They are frequently found in marine organisms [26]. These are more attractable heterocyclic structural framework to medicinal chemist [27], due to their broad spectrum of biological properties including (2S)cannabinoid receptor 2 (CB2) [28], immunosuppressive [29], muscarinic [30], α, β_3 -receptor antagonist [31], antimicrobial [32], insecticides [33], histamine-H3 [34], anti-inflammatory [35],

analgesic [36], diabetic, anticancer, antiparasitic and anti-helminthic properties. The US FDA approved drug such as ataluren (2), contains 1,2,4-oxadiazole framework as a part of the structure and is used for the treatment of muscular dystrophy [37–39].

The above biological findings and the continuous of demand for the new anticancer agents prompted us to design and synthesize a set of 1,2,4-oxadiazole linked 5-fluorouracil derivatives (7a–j). Their structures were confirmed by ^1H NMR, ^{13}C NMR and mass spectral data. Their anticancer activity towards four human cancer cells such as breast cancer (MCF-7, MDA MB-231), lung cancer (A549) and prostate cancer (DU-145) were evaluated.

Results and discussion

The synthesis of 1,2,4-oxadiazole linked 5-fluorouracil derivatives (7a–j) described in this study are outlined in Scheme 1. Commercially available 5-fluorouracil (1) was treated with 4-(bromomethyl)benzonitrile (3) in the presence of a base, DBU and anhydrous DMF at room



temperature for 12 h to give an intermediate compound **4** with 67% yield. The resulting nitrile intermediate **4** was reacted with hydroxylamine hydrochloride in triethyl amine base in methanol at reflux for 6 h to afford pure amidoxime compound **5** with 82% yield. Further, intermediate **5** was subjected to cyclization with substituted aromatic carboxylic acids (**6a–j**) in presence of a coupling reagent, EDC·HCl and a base sodium acetate in ethanol at reflux for 3 h to afford pure final compounds **7a–j** in good yields.

Experimental section

Materials and methods

All chemicals and reagents were obtained from Aldrich (Sigma-Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) and were used without further purification. Human cancer cell lines such as (MCF-7, MDA MB-231) were collected from Sigma-Aldrich. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254, and visualization on TLC was achieved by UV light or iodine indicator. ^1H and ^{13}C NMR spectra were recorded on Gemini Varian-VXR-unity (400 MHz, 300 MHz) instrument. Chemical shifts (δ) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. Melting points were determined with an electro thermal melting point apparatus and are uncorrected.

4-((5-Fluoro-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl)methyl)benzonitrile (**4**)

Compound **3** (14.9 g, 0.076 mol) was dissolved in 10 ml of dry DMF and added dropwise to a solution of 5-fluorouracil (**1**) (10 g, 0.076 mol) and DBU (12.6 ml, 0.084 mol) in dry DMF (40 ml) under N_2 atmosphere at 4 °C. The reaction mixture was allowed to stir at room temperature overnight, and then concentrated. The crude product was purified using flash chromatography (hexane/ethyl acetate = 1:1) to afford corresponding product **4** as 12.6 g with 67% yield. IR (KBr): 3265 (N–H stretching vibration), 3021 (Ar–C–H str.), 2893 (C–H str.), 1632 (NH(C=O)–C=C– stretching vibration of amide), 1562, 1469 (C=N and C=C stretching vibration of pyridine Nucleus), 988 (C–F stretching vibration), 782 (2-Substituted pyridine), ^1H -NMR (300 MHz, DMSO- d_6): δ 4.86 (s, 2H), 7.34 (d, 2H, $J = 8.0$ Hz), 7.72 (d, 2H, $J = 8.0$ Hz), 8.23 (d, 1H, $J = 6.1$ Hz), 11.89 (s, 1H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 156.8, 149.3, 141.6, 141.0, 134.3, 130.1, 126.1, 118.3, 109.1, 49.7; MS (ESI): m/z 246 [M+H] $^+$.

(1E)-4-((5-Fluoro-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl)methyl)-*N'*-hydroxybenzamidine (**5**)

To a solution of compound 4-((5-fluoro-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl)methyl)benzonitrile (**4**) (12 g, 0.049 mol) in methanol was added Et_3N (20 ml, 0.146 mol) and $\text{NH}_2\text{OH}\cdot\text{HCl}$ (10.2 g, 0.146 mol), and the reaction mixture was refluxed for 6 h. The reaction mixture was concentrated under vacuum; the residue was diluted with water and extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4 , concentrated in vacuo and purified by flash column chromatography on silica gel by using ethyl acetate/hexane (9:1) to afford the pure product **5** as 11.2 g with 82% yield. IR (KBr): 3314 (N–H stretching vibration), 3052 (Ar–C–H str.), 2962 (C–H str.), 1684 (NH(C=O)–C=C– stretching vibration of amide), 1684, 1485 (C=N and C=C stretching vibration of pyridine Nucleus), 974 (C–F stretching vibration), 766 (2-Substituted pyridine), ^1H NMR (300 MHz, DMSO- d_6): δ 4.82 (s, 2H), 5.88 (s, 2H), 7.26 (d, 2H, $J = 8.0$ Hz), 7.70 (d, 2H, $J = 8.0$ Hz), 8.22 (d, 1H, $J = 6.0$ Hz), 11.89 (s, 1H), 14.35 (s, 1H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 179.6, 156.7, 150.3, 141.3, 140.7, 134.1, 131.0, 129.3, 126.2, 49.2; MS (ESI): m/z 279 [M+H] $^+$.

1-(4-(5-Phenyl-1,2,4-oxadiazol-3-yl)benzyl)-5-fluoropyrimidine-2,4(1H,3H)-dione (**7a**)

A solution of substituted benzoic acid (**6a**) (0.16 ml, 0.0017 mol) in anhydrous CH_2Cl_2 (50 ml) was cooled to 0 °C and ethyl-(*N,N'*-dimethylamino)propylcarbodiimide hydrochloride (EDC·HCl) (418 mg, 0.00269 mol) was added to it under nitrogen atmosphere. The reaction mixture was stirred at 0 °C for an additional half an hour. To this mixture, (1E)-4-((5-fluoro-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl)methyl)-*N'*-hydroxybenzamidine (**5**) (500 mg, 0.0017 mmol) was added and stirring was continued for another half an hour at 0 °C. The reaction mixture was slowly allowed to attain room temperature with continuous stirring. Then it was heated to 110 °C for 2 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled down to the room temperature, to obtain the solid product, which was then filtered and washed with CH_2Cl_2 . The resultant white solid was heated for 3 h by dissolving it in a mixture of ethanol (20 ml), sodium acetate (83 mg, 0.0017 mmol) and water (3 ml). The reaction mixture was then cooled down to room temperature. The resultant solid was filtered and purified by recrystallization using ethanol to obtain the desired solid compound **7a** with 32% (210.4 mg) yield.

Mp: 316–318 °C, IR (KBr): 3176 (N–H stretching vibration), 2965 (Ar–C–H str.), 2944 (C–H str.), 1642 (NH(C=O)–C=C– stretching vibration of amide), 1598,

1425 (C=N and C=C stretching vibration of pyridine Nucleus), 1065 (C–F stretching vibration), 776 (2-Substituted pyridine), ¹H NMR (300 MHz, DMSO-d₆): δ 4.98 (s, 2H), 7.34–7.45 (m, 3H), 7.61 (d, 2H, J = 8.1 Hz), 7.94 (d, 2H, J = 7.6 Hz), 8.24 (d, 1H, J = 6.1 Hz), 8.28 (d, 2H, J = 8.1 Hz), 11.88 (s, 1H); ¹³C NMR (75 MHz, DMSO-d₆): δ 52.8, 127.5, 128.4, 128.7, 129.5, 130.4, 131.5, 131.8, 134.2, 134.7, 138.4, 140.3, 145.5, 151.7, 155.6, 157.8, 158.3, 163.7; MS (ESI): m/z 365 [M+H]⁺.

1-(4-(5-(3,4,5-trimethoxyphenyl)-1,2,4-oxadiazol-3-yl)benzyl)-5-fluoropyrimidine-2,4(1H,3H)-dione (7b)

The compound, **7b** was prepared following the method described for the preparation of the compound **7a**, employing **5** (500 mg, 0.0017 mol) with 3,4,5-trimethoxybenzoic acid (**6b**) (361 mg, 0.0017 mol), EDC·HCl (418 mg, 0.00269 mol) and sodium acetate (83 mg, 0.0017 mmol) to afford pure compound **7b**, 308.4 mg in 38% yield. Mp: 330–332 °C, IR (KBr): 3181 (N–H stretching vibration), 3062 (Ar–C–H str.), 2923 (C–H str.), 1657 (NH(C=O)–C=C– stretching vibration of amide), 1602, 1403 (C=N and C=C stretching vibration of pyridine Nucleus), 1093 (C–F stretching vibration), 773 (2-Substituted pyridine), ¹H NMR (300 MHz, DMSO-d₆): δ 3.88 (s, 3H), 3.91 (s, 6H), 4.90 (s, 2H), 7.63 (d, 2H, J = 8.0 Hz), 7.69 (s, 2H), 8.23 (d, 1H, J = 6.1 Hz), 8.28 (d, 2H, J = 8.0 Hz), 11.87 (s, 1H); ¹³C NMR (75 MHz, DMSO-d₆): δ 52.7, 57.6, 61.5, 106.4, 12.6, 128.5, 128.7, 129.4, 130.4, 131.5, 134.7, 138.2, 140.2, 143.5, 145.4, 151.7, 155.6, 155.8, 157.6, 158.4, 165.7; MS (ESI): m/z 455 [M+H]⁺.

1-(4-(5-(3,5-dimethoxyphenyl)-1,2,4-oxadiazol-3-yl)benzyl)-5-fluoropyrimidine-2,4(1H,3H)-dione (7c)

The compound, **7c** was prepared similar to the preparation of the compound **7a**, in which compound **5** (500 mg, 0.0017 mol) was treated with 3,5-dimethoxybenzoic acid (**6c**) (309 mg, 0.0017 mol), EDC·HCl (418 mg, 0.00269 mol) and sodium acetate (83 mg 0.0017 mol) to afford pure compound **7c**, 284.2 mg in 37% yield. Mp: 345–347 °C, IR (KBr): 3181 (N–H stretching vibration), 3065 (Ar–C–H str.), 2912 (C–H str.), 1621 (NH(C=O)–C=C– stretching vibration of amide), 1612, 1423 (C=N and C=C stretching vibration of pyridine Nucleus), 1053 (C–F stretching vibration), 762 (2-Substituted pyridine), ¹H NMR (300 MHz, DMSO-d₆): δ 3.79 (s, 6H), 4.98 (s, 2H), 6.98 (s, 1H), 7.61 (d, 2H, J = 8.1 Hz), 7.67 (s, 2H), 8.24 (d, 1H, J = 6.1 Hz), 8.29 (d, 2H, J = 8.1 Hz), 11.89 (s, 1H); ¹³C NMR (75 MHz, DMSO-d₆): δ 52.6, 57.6, 104.6, 105.7, 127.6, 128.4, 128.7, 130.4, 134.5, 134.8, 138.5, 140.2, 145.8, 151.7, 155.9, 157.4, 158.7, 162.6, 165.7; MS (ESI): m/z 425 [M+H]⁺.

1-(4-(5-(4-Methoxyphenyl)-1,2,4-oxadiazol-3-yl)benzyl)-5-fluoropyrimidine-2,4(1H,3H)-dione (7d)

The compound, **7d** was prepared following the method described for the preparation of the compound **7a**, employing **5** (500 mg, 0.0017 mol) with 4-methoxybenzoic acid (**6d**) (258 mg, 0.0017 mol), EDC·HCl (418 mg, 0.00269 mol) and sodium acetate (83 mg, 0.0017 mol) to afford pure compound **7d**, 291.3 mg in 41% yield. Mp: 342–344 °C, IR (KBr): 3181 (N–H stretching vibration), 3021 (Ar–C–H str.), 2895 (C–H str.), 1641 (NH(C=O)–C=C– stretching vibration of amide), 1618, 1386 (C=N and C=C stretching vibration of pyridine Nucleus), 1093 (C–F stretching vibration), 784 (2-Substituted pyridine), ¹H NMR (300 MHz, DMSO-d₆): δ 3.96 (s, 3H), 5.06 (s, 2H), 7.17 (d, 2H, J = 7.7 Hz), 7.63 (d, 2H, J = 8.1 Hz), 8.09 (d, 2H, J = 7.7 Hz), 8.24 (d, 1H, J = 6.1 Hz), 8.28 (d, 2H, J = 8.1 Hz), 11.89 (s, 1H); ¹³C NMR (75 MHz, DMSO-d₆): δ 52.8, 57.8, 118.4, 118.9, 127.4, 128.6, 128.9, 130.5, 134.5, 138.2, 140.6, 145.6, 151.8, 155.6, 157.3, 158.4, 162.5, 165.8; MS (ESI): m/z 395 [M+H]⁺.

1-(4-(5-(4-Chlorophenyl)-1,2,4-oxadiazol-3-yl)benzyl)-5-fluoropyrimidine-2,4(1H,3H)-dione (7e)

This compound, **7e** was prepared following similar method used for the preparation of the compound **7a**, employing **5** (500 mg, 0.0017 mol) with 4-chlorobenzoic acid (**6e**) (266 mg, 0.0017 mol), EDC·HCl (418 mg, 0.00269 mol) and sodium acetate (83 mg, 0.0017 mol) to afford pure compound **7e**, 313.5 mg in 44% yield. Mp: 338–340 °C, IR (KBr): 3163 (N–H stretching vibration), 3075 (Ar–C–H str.), 2897 (C–H str.), 1691 (NH(C=O)–C=C– stretching vibration of amide), 1610, 1422 (C=N and C=C stretching vibration of pyridine Nucleus), 1094 (C–F stretching vibration), 794 (2-Substituted pyridine), ¹H NMR (300 MHz, DMSO-d₆): δ 5.19 (s, 2H), 7.61 (d, 2H, J = 8.1 Hz), 7.72 (d, 2H, J = 7.8 Hz), 8.12 (d, 2H, J = 7.8 Hz), 8.25 (d, 1H, J = 6.2 Hz), 8.30 (d, 2H, J = 8.1 Hz), 11.89 (s, 1H); ¹³C NMR (75 MHz, DMSO-d₆): δ 53.2, 127.6, 128.7, 128.9, 129.5, 130.4, 130.8, 133.4, 134.5, 134.7, 138.5, 140.3, 145.6, 151.7, 155.6, 157.4, 158.7, 165.8; MS (ESI): m/z 399 [M+H]⁺.

1-(4-(5-(4-Bromophenyl)-1,2,4-oxadiazol-3-yl)benzyl)-5-fluoropyrimidine-2,4(1H,3H)-dione (7f)

The compound **7f** was prepared using the method described for the preparation of the compound **7a**, employing **5** (500 mg, 0.0017 mol) with 4-bromobenzoic acid (**6f**) (341 mg, 0.0017 mol), EDC·HCl (418 mg, 0.00269 mol) and sodium acetate (83 mg, 0.0017 mol) to afford pure compound **7f**, 372.4 mg in 47% yield. Mp: 362–364 °C, IR (KBr): 3203 (N–H stretching vibration), 3055 (Ar–C–H str.), 2965 (C–H str.), 1643 (NH(C=O)–C=C– stretching vibration of amide), 1632, 1403 (C=N

and C=C stretching vibration of pyridine Nucleus), 1093 (C–F stretching vibration), 769 (2-Substituted pyridine), ¹H NMR (300 MHz, DMSO-d₆): δ 5.17 (s, 2H), 7.64 (d, 2H, J = 8.1 Hz), 7.76–7.88 (m, 4H), 8.23 (d, 1H, J = 6.2 Hz), 8.31 (d, 2H, J = 8.1 Hz), 11.89 (s, 1H); ¹³C NMR (75 MHz, DMSO-d₆): δ 52.9, 126.7, 127.6, 128.7, 128.9, 129.2, 130.6, 133.4, 134.5, 134.8, 138.6, 140.6, 145.8, 151.6, 155.6, 157.8, 158.9, 165.7; MS (ESI): m/z 445 [M+H]⁺.

1-(4-(5-(4-Nitrophenyl)-1,2,4-oxadiazol-3-yl)benzyl)-5-fluoropyrimidine-2,4(1H,3H)-dione (7g)

The compound, **7g** was prepared as described above, employing **5** (500 mg, 0.0017 mol) with 4-nitrobenzoic acid (**6g**) (284 mg, 0.0017 mol), EDC·HCl (418 mg, 0.00269 mol) and sodium acetate (83 mg, 0.0017 mol) to afford pure compound **7g**, 367.3 mg in 50% yield. Mp: 370–372 °C, IR (KBr): 3176 (N–H stretching vibration), 2906 (Ar–C–H str.), 2966 (C–H str.), 1668 (NH(C=O)–C=C– stretching vibration of amide), 1631, 1396 (C=N and C=C stretching vibration of pyridine Nucleus), 1112 (C–F stretching vibration), 791 (2-Substituted pyridine), ¹H NMR (300 MHz, DMSO-d₆): δ 5.28 (s, 2H), 7.65 (d, 2H, J = 8.2 Hz), 7.96 (d, 2H, J = 8.1 Hz), 8.14 (d, 2H, J = 8.1 Hz), 8.24 (d, 1H, J = 6.2 Hz), 8.33 (d, 2H, J = 8.2 Hz), 11.91 (s, 1H); ¹³C NMR (75 MHz, DMSO-d₆): δ 53.5, 126.7, 127.3, 127.8, 128.4, 128.7, 130.5, 134.7, 138.4, 139.6, 140.6, 145.8, 149.4, 151.7, 155.6, 157.3, 158.4, 165.8; MS (ESI): m/z 410 [M+H]⁺.

1-(4-(5-(3,5-Dinitrophenyl)-1,2,4-oxadiazol-3-yl)benzyl)-5-fluoropyrimidine-2,4(1H,3H)-dione (7h)

The compound **7h** was prepared as per the method used for the preparation of the compound **7a**, in which compound **5** (500 mg, 0.0017 mol) treated with 3,5-dinitrobenzoic acid (**6h**) (360 mg, 0.0017 mol), EDC·HCl (418 mg, 0.00269 mol) and sodium acetate (83 mg, 0.0017 mol) to afford pure compound **7h**, 342.8 mg in 42% yield. Mp: 376–378 °C, IR (KBr): 3206 (N–H stretching vibration), 3112 (Ar–C–H str.), 2887 (C–H str.), 1648 (NH(C=O)–C=C– stretching vibration of amide), 1603, 1403 (C=N and C=C stretching vibration of pyridine Nucleus), 1114 (C–F stretching vibration), 769 (2-Substituted pyridine), ¹H NMR (300 MHz, DMSO-d₆): δ 5.37 (s, 2H), 7.66 (d, 2H, J = 8.3 Hz), 8.25 (d, 1H, J = 6.3 Hz), 8.37 (d, 2H, J = 8.3 Hz), 8.52 (s, 2H), 8.89 (s, 1H), 11.91 (s, 1H); ¹³C NMR (75 MHz, DMSO-d₆): δ 53.7, 121.6, 127.6, 128.5, 128.9, 129.3, 130.7, 134.5, 138.6, 138.9, 140.4, 145.8, 149.7, 151.7, 155.8, 157.7, 158.7, 165.9; MS (ESI): m/z 455 [M+H]⁺.

1-(4-(5-p-Tolyl-1,2,4-oxadiazol-3-yl)benzyl)-5-fluoropyrimidine-2,4(1H,3H)-dione (7i)

The compound **7i** was prepared as described above. In this, compound **5** (500 mg, 0.0017 mol) was reacted with 4-methylbenzoic acid (**6i**) (231 mg, 0.0017 mmol), EDC·HCl (418 mg, 0.00269 mol) and sodium acetate (83 mg, 0.0017 mol) to afford pure compound **7i**, 239.6 mg in 35% yield. Mp: 356–358 °C, IR (KBr): 3224 (N–H stretching vibration), 3102 (Ar–C–H str.), 2986 (C–H str.), 1643 (NH(C=O)–C=C– stretching vibration of amide), 1632, 1405 (C=N and C=C stretching vibration of pyridine Nucleus), 1087 (C–F stretching vibration), 756 (2-Substituted pyridine), ¹H NMR (300 MHz, DMSO-d₆): δ 2.69 (s, 3H), 5.10 (s, 2H), 7.37 (d, 2H, J = 7.8 Hz), 7.62 (d, 2H, J = 8.1 Hz), 7.86 (d, 2H, J = 7.8 Hz), 8.23 (d, 1H, J = 6.1 Hz), 8.31 (d, 2H, J = 8.1 Hz), 11.89 (s, 1H); ¹³C NMR (75 MHz, DMSO-d₆): δ 35.8, 52.7, 127.5, 128.7, 128.9, 129.5, 130.5, 131.7, 133.4, 134.8, 138.6, 140.6, 141.8, 145.7, 151.7, 157.8, 158.9, 165.6; MS (ESI): m/z 379 [M+H]⁺.

1-(4-(5-(4-Bromo-3,5-dinitrophenyl)-1,2,4-oxadiazol-3-yl)benzyl)-5-fluoropyrimidine-2,4(1H,3H)-dione (7j)

This compound **7j** was prepared following the method described for the preparation of the compound **7a**, in which compound **5** (500 mg, 0.0017 mol) was made to react with 4-bromo-3,5-dinitrobenzoic acid (**6j**) (494 mg, 0.0017 mol), EDC·HCl (418 mg, 0.00269 mol) and sodium acetate (83 mg, 0.0017 mol) to afford pure compound **7j**, 356.9 mg in 37% yield. Mp: 386–388 °C, IR (KBr): 3125 (N–H stretching vibration), 3098 (Ar–C–H str.), 2995 (C–H str.), 1664 (NH(C=O)–C=C– stretching vibration of amide), 1609, 1409 (C=N and C=C stretching vibration of pyridine Nucleus), 1076 (C–F stretching vibration), 748 (2-Substituted pyridine), ¹H NMR (300 MHz, DMSO-d₆): δ 5.40 (s, 2H), 7.65 (d, 2H, J = 8.3 Hz), 8.25 (d, 1H, J = 6.3 Hz), 8.37 (d, 2H, J = 8.3 Hz), 8.68 (s, 2H), 11.92 (s, 1H); ¹³C NMR (75 MHz, DMSO-d₆): δ 53.7, 122.7, 27.6, 128.5, 128.9, 130.6, 132.6, 134.8, 136.7, 138.4, 140.6, 145.8, 151.7, 157.6, 158.9, 166.8; MS (ESI): m/z 534 [M+H]⁺.

Biological evaluation

In vitro cytotoxicity

The target compounds (**7a–j**) were examined for their anticancer activity against a panel of four human cancer cell lines including breast cancer (MCF-7, MDA MB-231), lung cancer (A549) and prostate cancer (DU-145) by using MTT method. Etoposide was used as reference standard and the obtained results were summarized in Table 1.

Among the compounds (**7a–j**) synthesized **7a**, **7b**, **7c**, **7d** and **7i** possessed good activity with IC₅₀ values

Table 1 Anticancer activity of newly synthesized compounds **7a–j** with IC₅₀ in μM

Compound	MCF-7	A549	DU-145	MDA MB-231
7a	0.76 ± 0.044	0.18 ± 0.019	1.13 ± 0.55	0.93 ± 0.013
7b	0.011 ± 0.009	0.053 ± 0.0071	0.017 ± 0.0062	0.021 ± 0.0028
7c	0.88 ± 0.073	1.44 ± 0.32	1.28 ± 0.27	1.95 ± 0.19
7d	1.78 ± 0.22	1.67 ± 0.49	2.10 ± 1.09	2.34 ± 1.10
7e	3.45 ± 1.87	6.34 ± 3.24	ND	3.98 ± 1.88
7f	5.98 ± 2.56	ND	6.22 ± 2.91	ND
7g	9.22 ± 5.66	10.5 ± 5.72	4.33 ± 4.25	2.75 ± 1.24
7h	8.21 ± 5.19	11.3 ± 6.32	ND	ND
7i	2.17 ± 1.66	1.88 ± 0.25	2.65 ± 1.26	2.14 ± 0.94
7j	7.12 ± 4.30	13.6 ± 7.56	ND	19.4 ± 8.11
Etoposide	2.11 ± 0.024	3.08 ± 0.135	1.97 ± 0.45	1.91 ± 0.84

ND not determined

ranging from 0.011 ± 0.009 to 19.4 ± 8.11 μM and standard reference showed IC₅₀ values range from 1.91 ± 0.84 to 3.08 ± 0.135 μM , respectively. The structure–activity relationship (SAR) studies indicated that the compound **7a** without substituent on the phenyl ring attached to 1,2,4-oxadiazole moiety has showed good anticancer activity against four cell lines (MCF-7 = 0.76 ± 0.044 μM ; A549 = 0.18 ± 0.019 μM ; DU145 = 1.13 ± 0.55 μM and MDA MB-231 = 0.93 ± 0.013 μM). Up on introduction of electron-donating 3,4,5-trimethoxy group on the phenyl ring resulted compound **7b**, showed more significant anticancer activity (MCF-7 = 0.011 ± 0.009 μM ; A549 = 0.053 ± 0.0071 μM ; DU145 = 0.017 ± 0.0062 μM and MDA MB-231 = 0.021 ± 0.0028 μM) than standard against cancer cells presented in Table 1. When one methoxy group is removed, resulting compound **7c** displayed slightly decreased activity on four cell lines (MCF-7 = 0.88 ± 0.073 μM ; A549 = 1.44 ± 0.32 μM ; DU145 = 1.28 ± 0.27 μM and MDA MB-231 = 1.95 ± 0.19 μM) when compared with **7b**. Where, compound **7d** with 4-methoxy substituent has showed reduced anticancer activity (MCF-7 = 1.78 ± 0.22 μM ; A549 = 1.67 ± 0.49 μM ; DU145 = 2.10 ± 1.09 μM and MDA MB-231 = 2.34 ± 1.10 μM) than **7c**. Instead of 4-methoxy group with weak electron-donating 4-methyl group (**7i**) exhibited acceptable activity (MCF-7 = 2.17 ± 1.66 μM ; A549 = 1.88 ± 0.25 μM ; DU145 = 2.65 ± 1.26 μM and MDA MB-231 = 2.14 ± 0.94 μM). Further, the compounds **7e**, **7f**, **7g**, **7h** and **7j**, which possess electron-withdrawing substituents on the phenyl ring, showed moderate activities compared to those compounds **7a**, **7b**, **7c**, **7d** and **7i**, without electron withdrawing and with electron-donating substituents.

Molecular docking

The docking studies of the potent 1,2,4-Oxadiazole linked 5-Fluorouracil derivatives (**7a–7j**) were performed using Molegro Virtual Docker (MVD). The crystal structure of Human VEGFR-2 enzyme (PDB ID: 1YWN) along with the crystal ligand imatinib was downloaded from protein databank [40]. Human VEGFR-2 enzyme is the key enzyme in angiogenesis, hematopoiesis and vasculogenic. All the chemical structures were prepared by using Marvin sketch and minimized and saved in a single file as SDF format. MVD was used to perform computational studies, cavity prediction, assigning bond orders, defining the active binding sites of the Human VEGFR-2 enzyme, structure refinement and preparation. The protein preparation was carried out with MVD and the chain was treated to add missing hydrogen, assign proper bond orders and deleted water molecules. The structure output format was set to pose viewer file so as to view the output of resulting docking studies and hydrogen bond interactions of different poses with the protein. The 2D and 3D interactions were generated by using Discovery Studio Visualizer.

In silico ADMET prediction

In silico ADMET screened for 5-fluoro-1-(4-(5-substituted phenyl-1,2,4-oxadiazol-3-yl)benzyl)pyrimidine-2,4(1H,3H)-dione derivatives (**7a–7j**) was assessed by using DataWarrior Software [41]. It calculates the properties of the datasets of ligands to determine the violation of Lipinski's rule of 5 and toxicity parameters. All the calculated chemical properties are represented in Table 2.

Molecular docking studies

The docking studies of the potent compounds **7a–7j** were performed using Molegro Virtual Docker (MVD). The crystal structure of human Vascular Endothelial Growth Factor Receptors (VEGFR-2) enzyme (PDB ID: 1YWN) along with the co-crystal ligand was downloaded from protein databank. Design of VEGFR-2 could be potential target for inhibition of blood vessel formation and leads to the development of anti-angiogenesis agents. All the chemical structures were prepared by using Marvin sketch and minimized. Mol file format structures were converted into Mol2 file format by using Discovery studio. MVD was used to perform computational studies, cavity prediction, assigning bond orders, structure refinement, defining the active binding sites of the VEGFR-2 and structure preparation. The protein preparation was carried out with MVD and the chain was treated to add missing hydrogen, assign proper bond orders and deleted water molecules. The structure output format was set to pose viewer file so

Table 2 In silico ADMET prediction of 1,2,4-oxadiazole linked 5-fluorouracil derivatives (**7a–7j**)

Compd	Total Molweight	cLogP	cLogS	HAA	HDA	TSA	Nrotb	TPSA	Druglikeness	Mutagenic	Tumorigenic	Reproductive effective	Irritant
7a	364.3	2.40	-6.49	7	1	268.57	4	88.33	-1.025	None	None	None	None
7b	454.4	2.19	-6.54	10	1	335.35	7	116.02	-0.96	None	None	None	None
7c	424.3	2.26	-6.56	9	1	313.09	6	106.79	-0.96	None	None	None	None
7d	394.3	2.33	-6.50	8	1	290.83	5	97.56	-0.96	None	None	None	None
7e	398.7	3.00	-7.22	7	1	283.99	4	88.33	-0.97	None	None	None	None
7f	443.2	3.12	-7.32	7	1	287.2	4	88.33	-2.81	None	None	None	None
7g	409.3	1.48	-6.95	10	1	292.24	5	134.15	-6.03	None	None	None	None
7h	454.3	0.56	-7.41	13	1	315.91	6	179.97	-6.03	None	None	None	None
7i	378.3	2.74	-6.83	7	1	280.83	4	88.33	-1.05	None	None	None	None
7j	533.2	1.28	-8.24	13	1	334.54	6	179.97	-7.82	None	None	None	None

as to view the output of resulting docking studies and hydrogen bond interactions of different poses with the protein. The 2D interactions were generated from Protein Plus and 3D interactions were generated from MVD.

In the present study, the synthesized compounds were docked into X-ray crystal structures of Human VEGFR-2 enzyme (PDB ID: 1YWN) to understand the possible target mechanism of action. The cavities were detected with MVD and the following are the active residues involved with co-crystal ligand, as it forms hydrogen bond interactions with Glu883, Glu915, Cys917, Asp1044, Arg1049 residues and hydrophobic interactions with Leu838, Val846, Val897, Val914, Cys1043, and Asp1044, residues. The docking has validated and found same interactions with Moldock score -199.37 and H-bond energy -6.24 kcal/mol (Fig. 2).

All the synthesized compounds form hydrogen bond interactions with Asp1044 and have shown same interaction with crystal ligand. The binding affinity of the docked compounds were expressed in negative binding energy kcal/mol (Moldock score). The ligands with more negative value of Moldock score will have more affinity with protein binding. 3,5-dinitro substituted compounds (7j and 7h) established a hydrogen bond (NH—ON) with amino group of Asp1044 and oxygen atom of oxadiazole (NH—O) with amino group of Lys866 with a H-bond energy -7.40 and -7.34 , respectively and with Moldock score values are -156.20 and -157.88 , respectively. The 7j and 7h

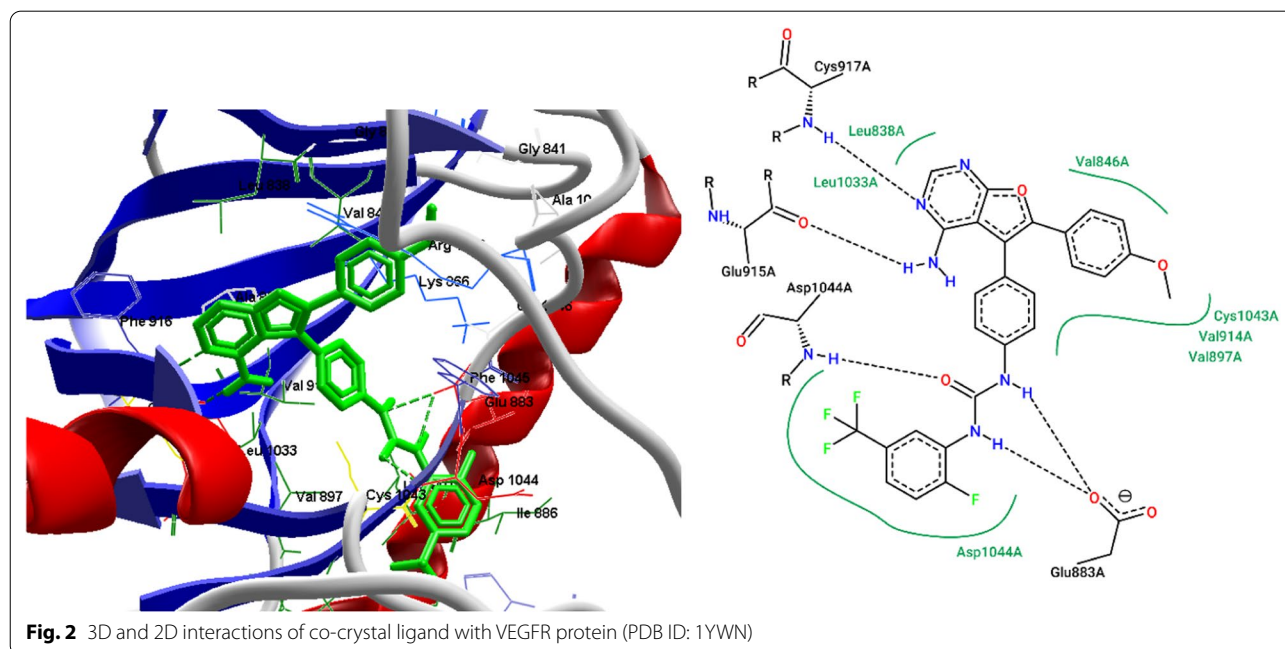
also form same hydrophobic interactions with Arg840, Val846, Asp1044, Gly1046, and Arg1049 amino acid residues (Figs. 3, 4). Compound 7c oxygen of oxadiazole group forms a hydrogen bond (NH—O) with amino hydrogen of Gly1046 with a H-bond energy of -2.59 kcal/mol (Fig. 5) and hydrophobic interactions with Val846, Arg840, Gly1046, Arg1049 amino acid residues. All the docked ligands have exhibited same interactions with active site of VEGFR. From the data it is revealed that compounds of the series 7j (Moldock score -156.20 kcal/mol) and 7h (Moldock score -157.88 kcal/mol) showed good inhibitory constant and excellent free energy of binding, which might be the reason for anticancer activity.

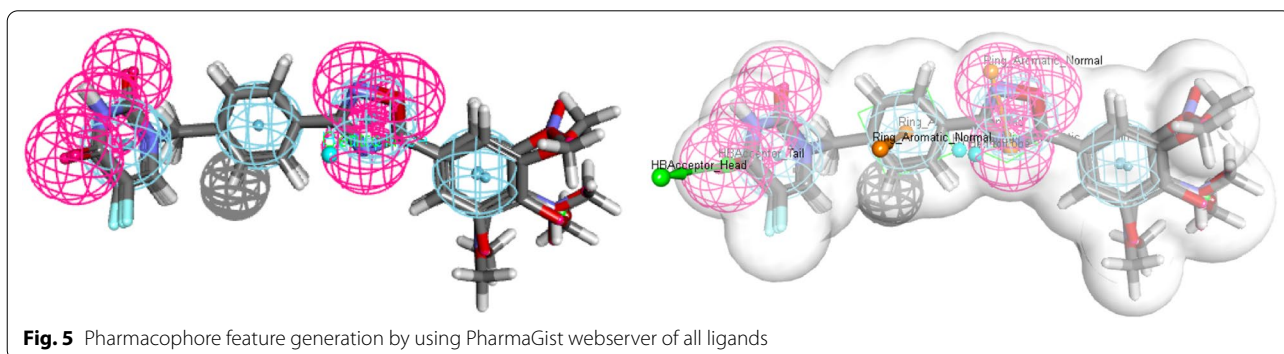
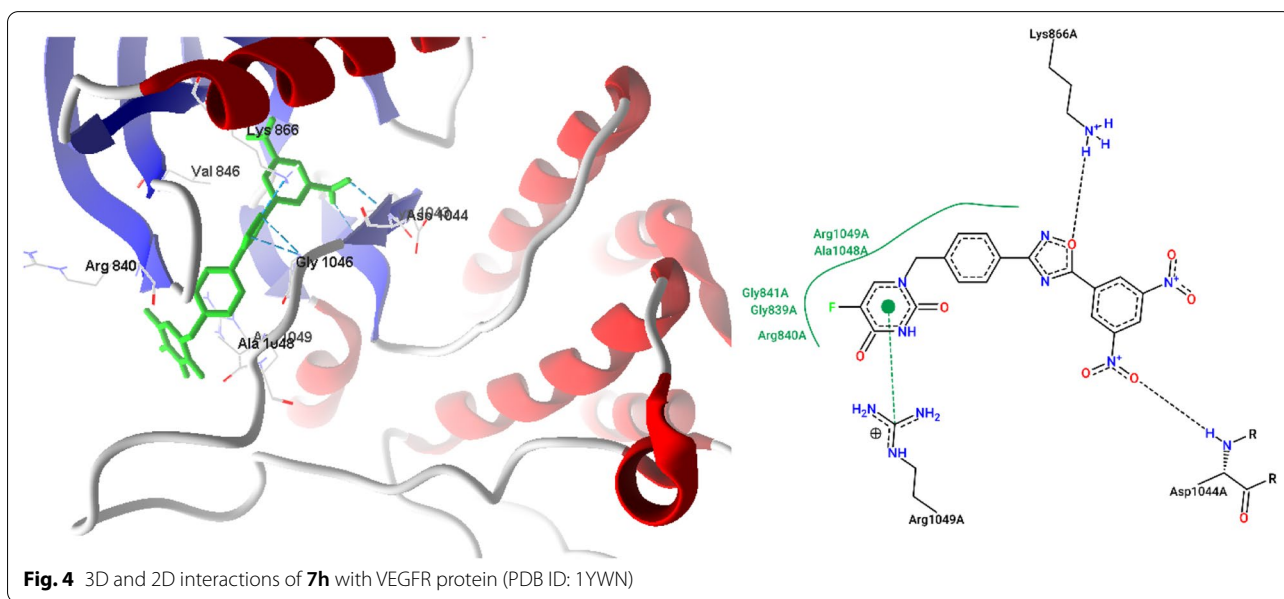
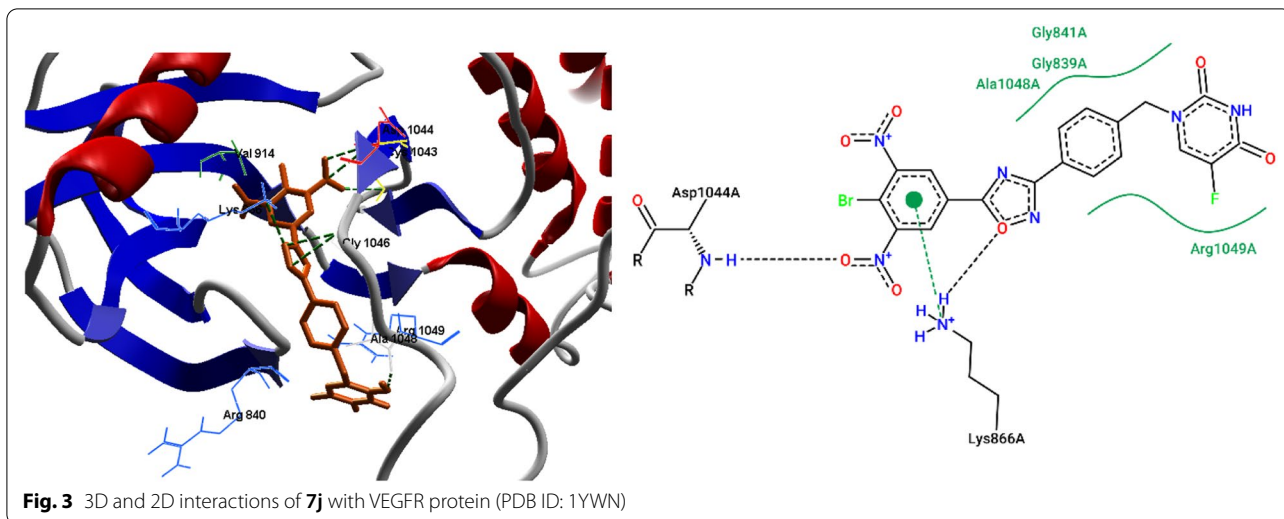
Pharmacophore prediction

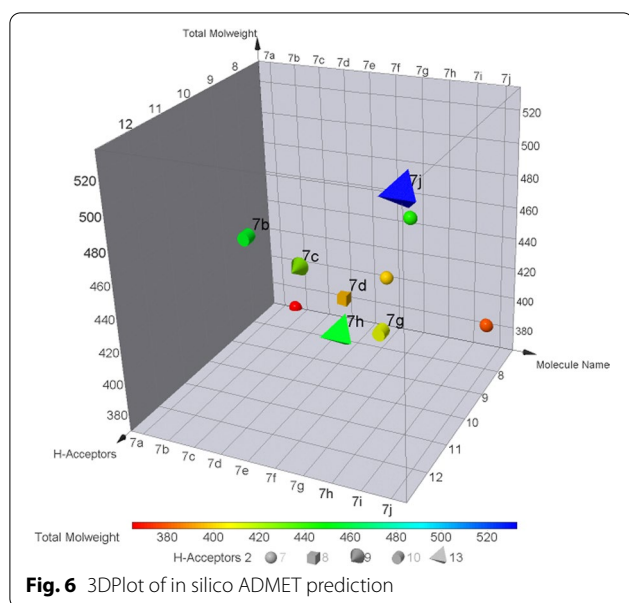
All the synthesized compounds generated pharmacophore features by using PharmaGist web server [42]. It will predict the spatial arrangement of features like hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), hydrophobic center (HY) and positive ionisable (PI) which are essential for a ligand to interact with specific target protein. All compounds were submitted to PharmaGist and the pharmacophoric score is found to be 75.17 with 10 Spatial Features, 4 Aromatic, 1 Donor and 5 Acceptor features for all set of ligands.

In silico ADMET properties

The in silico ADMET properties of 5-fluoro-1-(4-(5-substituted phenyl-1,2,4-oxadiazol-3-yl)benzyl)







pyrimidine-2,4(1H,3H)-dione derivatives (7a–7j) was assessed by using DataWarrior Software (Fig. 6). All the compounds were determined molecular descriptors for Rule of 5 (Lipinski rule) which states the oral bio-availability and drug like properties. The determinants

possess Molecular weight ≤ 500 , no of H-Acceptors ≤ 10 , no of H-Donor ≤ 5 . Among the calculated chemical descriptors, all the ligands have passed the Lipinski rule which states ligands did not violate more than one Rule of 5, except compound 7j substituted with 4-bromo-3,5-dinitro has violated Molecular weight and H-Acceptor.

MTT assay

Individual wells of a 96-well tissue culture micro titer plate were inoculated with 100 μ l of complete medium containing 1×10^4 cells. The plates were incubated at 37 °C in a humidified 5% CO₂ incubator for 18 h prior to the experiment. The results are shown in Table 3.

Conclusion

In conclusion, we have synthesized a library of 1,2,4-oxadiazole linked 5-fluorouracil derivatives (7a–j) and all these compounds were characterized by ¹HNMR, ¹³CNMR and Mass spectral analysis. Further, these compounds were investigated for their anticancer activity towards a panel of four human cancer cell lines such as (MCF-7, MDA MB-231), lung cancer (A549) and prostate cancer (DU-145) by using MTT method. Among them, compounds 7a, 7b, 7c, 7d and 7i were demonstrated more promising anticancer activity than

Table 3 Docking score and binding interactions of compounds with VEGFR kinase enzyme into the active site of 1YWN

S. no	Compound	Mol.dock score	Rerank score	H-bond (kcal/mol)	Interactions	
					H-bonding	Steric
1	7a	– 147.97	– 121.95	– 5.01	Lys866, Arg1030	Lys866, Val912, Gly920
2	7b	– 123.29	– 102.36	– 5.01	Lys866, Cys1043, Gly1046	Lys866, Cys1043, Gly1046, Ala1048, Arg1049
3	7c	– 151.14	– 111.31	– 2.59	Lys866, Gly1046	Val846, Arg840, Gly1046, Arg1049
4	7d	– 135.85	– 113.49	– 5.20	Lys866, Gly1046	Arg840, Lys866, Gly1046, Arg1049
5	7e	– 140.55	– 107.94	– 1.03	Arg1030	Val912, Val914, Gly920, Phe1045, Arg1049
6	7f	– 126.70	– 99.40	– 2.44	Arg1030	Val912, Val914, Ile913, Gly920, Arg1030, Phe1045
7	7g	– 124.66	– 98.82	– 2.30	Arg921, Cys1043	Asn921, Cys1043, Phe1045
8	7h	– 157.88	– 116.87	– 7.34	Lys866, Asp1044, Gly1046	Val846, Arg840, Lys866, Cys1043, Asp1044, Ala1048, Arg1049, Gly1046
9	7i	– 141.45	– 113.61	– 2.84	Arg1030	Val912, Asn921, Arg1030, Phe1045, Arg1049
10	7j	– 156.20	– 123.62	– 7.40	Lys866, Cys1043, Asp1044, Gly1046	Arg840, Lys866, Val846, Asp1044, Gly1046, Ala1048, Arg1049
11	4-amino-furo[2,3-d]pyrimidine	– 199.37	156.11	– 6.24	Glu883, Glu915, Cys917, Asp1044, Arg1049	Val846, Val897, Val914, Cys1043, Asp1044

standard. Further docking studies of all the compounds were presented, where the designed compounds showed good anticancer activity and excellent free energy of binding interactions with VEGFR-2 kinase domain.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13065-021-00757-y>.

Additional file 1: Spectra of synthesized compounds.

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Authors' contributions

RK and SK carried out the experimental work. LE performed the statistical analysis and drafted the manuscript. RR performed docking studies. All authors have gone through the final manuscript and approved it. All authors read and approved the final manuscript.

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All data generated or analyzed during this study are included in this published article (and its Additional file 1).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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