ARTICLE

Synthesis, Evaluation and Molecular Docking Studies of Some Novel Pyrazolo[3,4-d]pyrimidine Derivatives of 7-Methoxy Quinoline

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ABSTRACT

Pyrazolo[3,4-d]pyrimidine derivatives were synthesized from 4-hydrazino-7-methoxy quinoline (**2**) with ethoxymethylenecyanoacetate afforded ethyl 5-amino-1-(7-methoxyquinolin-4-yl)-1*H*-pyrazole-4-carboxylate (**3**). The compound **3** was hydrolyzed to get 5-amino-1-(7-methoxyquinolin-4-yl)-1*H*-pyrazole-4-carboxylic acid (**4**) and then reacted with acetic anhydride to afford 1-(7-methoxyquinolin-4-yl)-6-methylpyrazolo[3,4-d][1,3]oxazin-4(1*H*)-one (**5**), which was condensed with different aromatic amines to give a series of 5-substituted 1-(7-methoxyquinolin-4-yl)-6-methyl-5-aryl-1,5-dihydro-4*H*-pyrazolo[3,4-d]pyrimidin-4-one (**6**). The newly synthesized compounds were characterized and evaluated for their antibacterial and antioxidant activity,and molecular docking studies.

KEYWORDS

4-Hydrazino-7-methoxy quinolines, Pyrazolopyrimidines, Antimicrobial, Antioxidant, Molecular docking.

INTRODUCTION

Quinoline derivatives have been well known in medicinal chemistry because of their wide occurrence in various natural products, especially in alkaloids. The quinoline skeleton is used for many valuable synthetic agrochemicals and to design many synthetic compounds with diverse pharmacological activities [1]. Most of the pyrazoles, thiophenes and pyrimidines are highly bioactive and are widely used in pharmaceutics. A tremendous interest in the pyrazolo[3,4-d]pyrimidine derivatives has been observed and identified as a general class of adenosine receptors [2,3]. Our literature survey showed that the chemistry of fused pyrazolo[3,4-d]pyrimidine derivatives has drawn great attention due to their pharmacological importance [4,5] and their structural resemblance to purines [6]. In fact, several pyrazolo[3,4-d]pyrimidine derivatives demonstrated significant antimicrobial [7,8] and cytotoxic activities [9].

Many biologically active compounds found in the literature have pyrimidine, pyrazole or quinoline constituents in their structures [10-13]. Pyrimidoquinolines are important

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compounds because of their biological properties such as antimalarial [14], antimicrobial [15,16] and anti-inflammatory [17,18] activities. Furthermore, many synthetic pharmacophores possessing antibacterial [19], antifungal [20] and antimycotic [21] activities are based on the pyrimidyl structures. The development of pyrimidine-based antitumour [22] and antiviral [23] drugs have inspired chemists all over the world to prepare new pyrimidine-based compounds and to study their biological activities.

Prompted by the varied biological activities of pyrazolo-[3,4-d]pyrimidine derivatives and methoxyqinoline bearing compounds, we envisioned our approach toward the synthesis of a novel series of pyrazolo-[3,4-d]pyrimidine derivatives methoxy quinoline moiety and screened them for antibacterial and antioxidant activity. Hence, the *in silico* molecular docking studies of newly synthesized compounds were carried out to predict the tyrosine kinase (RTK) inhibitory activity. Toremifene drug was used as standard for our docking studies, which is known to be potential inhibitor of human estrogen receptor. The drugs which are currently used for the treatments of breast cancer were tamoxifen, raloxifene, toremifene [24]. Ingestion of this drug was based on interfering with either estrogen production or estrogen action which causes so many side effects such as blood clots, strokes, uterine cancer or cataracts [25]. The side effects of these drugs make the need for the necessity of new improved drugs.In the present study an attempt was made to evaluate their antibacterial property so we selected human estrogen receptor, tyrosine kinase (RTK) which is involved in causing breast cancer.

EXPERIMENTAL

Melting points were determined by open capillary method and areuncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded ($CDCl_3$) on a Bruker (400 MHz) using TMS as internal standard. Chemical shift values are given in δ (ppm) scales. The mass spectra were recorded on a JEOL JMS-D 300 spectrometer operating at 70 eV. Elemental analyses were performed on a Flash EA 1112 series CHNS-O Analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminium sheets (silica gel 60 F254) obtained from Merck. Commercial grade solvents and reagents were used without purification.

Synthesis of 4-hydrazino-7-methoxy quinoline (2): To the compound 4-chloro-7-methoxy quinolone (**1**) (25 g, 0.1 mol) in 100 mL ethanol, hydrazine hydrate (10 mL) was added. After being stirred at 80 °C for 8 h in the absence of light, the reaction mixture was diluted with water. The resulting precipitate were collected and recrystallized from ethanol to give light yellow needles state of compound. Yield 82.5 %.

Synthesis of ethyl 5-amino-1-(7-methoxyquinolin-4-yl)-1*H*-pyrazole-4-carboxylate (3): Ethoxymethylenecyanoacetate (22 g, 0.01 mol) was carefully added in small portions to (25 g, 0.01 mol) of 4-hydrazino-7-methoxy quinoline. The reaction was heated at 80 °C for 1 h in a water bath. The, the completion of the reaction was monitored by TLC. Upon completion, the reaction was cooled to room temperature, diluted with 100 mL water, stirred for 2 h. The solid obtained was filtered, washed with water and dried *in vacuo*. Crystallization from ethanol gave compound **3** as colourless crystalline solid.

Yield 62.5 %. ^{1}H NMR ($CDCl_3$): δ 1.29 (s, 3H, CH_3), 4.0 (s, 3H, OCH_3), 4.22-4.27 (d, 2H, CH_2), 6.71 (d, 2H, NH_2), 7.50-7.51 (d, 1H, methoxyquinoline), 7.53-7.53 (d, 1H, pyrazole), 7.78-7.82 (d, 1H, methoxyquinoline), 7.94 (s, 1H, methoxyquinoline), 7.94-7.97 (d, 1H, methoxyquinoline), 9.19-9.20 (d, 1H, methoxyquinoline). ^{13}C NMR (DMSO-d_6): 14.98, 56.71, 59.66, 94.97, 102.98, 117.19, 119.87, 122.40, 127.21, 142.91, 145.94, 146.43, 147.77, 152.29, 163.16, 163.61. LCMS: *m/z* = 313 (M+1). Anal. calcd. for $C_{16}H_{16}N_4O_3$: C-61.53, H-5.16, N-17.94 %; Found C-61.51, H-5.18, N-17.92 %.

Synthesis of 5-amino-1-(7-methoxyquinolin-4-yl)-1*H*-pyrazole-4-carboxylic acid (4): A mixture of ethyl 5-amino-1-(7-methoxyquinolin-4-yl)-1*H*-pyrazole-4-carboxylate (**3**) (20 g, 0.065 mol) and sodium hydroxide (3.8 g, 0.1 mol) was dissolved in 75 mL of methanol and 25 mL water. The contents were heated to reflux on a water bath for 5 h and the completion of the reaction was monitored by TLC. The reaction mass was cooled to room temperature and poured into 500 mL ice water, then adjusting pH to 4 using conc. HCl. The solids obtained were filtered, washed with water and crystallized from ethanol. ^{1}H NMR ($CDCl_3$): δ 1.29 (s, 3H, CH_3), 4.00 (s, 3H, OCH_3), 6.69 (d, 2H, NH_2), 7.49-7.56 (d, 1H, methoxyquinoline), 7.84-7.84 (d, 1H, pyrazole), 7.85-7.86 (d, 1H, methoxyquinoline), 7.92 (s, 1H, methoxyquinoline), 8.03-8.05 (d, 1H, methoxyquinoline), 9.21-9.22 (d, 1H, methoxyquinoline). ^{13}C NMR (DMSO-d_6): 56.78, 95.67, 102.14, 116.97, 119.84, 122.59, 127.60, 143.53, 145.02, 146.99, 147.25, 152.51, 163.43, 163.28. LCMS: *m/z* = 285.2 (M+1). Anal. calcd. for $C_{14}H_{12}N_4O_3$: C-59.15, H-4.25, N-16.88 %; Found C-59.17, H-4.22, N-16.88 %.

Synthesis of 1-(7-methoxyquinolin-4-yl)-6-methylpyrazolo[3,4-d][1,3]oxazin-4(1*H*)-one (5): 5-Amino-1-(7-methoxyquinolin-4-yl)-1*H*-pyrazole-4-carboxylic acid (**4**) (20 g 0.07 mol) taken in 40 mL acetic anhydride was heated to reflux on an oil bath for 8 h. It was then cooled to room temperature and poured into ice-cold water with stirring for 0.5 h. The solid was filtered, washed with water and then crystallized from ethyl acetate.

^{1}H NMR ($CDCl_3$): δ 2.39 (s, 3H, CH_3), 3.94 (s, 3H, OCH_3), 7.29-7.30 (d, 1H, methoxyquinoline), 7.32-7.32 (d, 1H, pyrazole), 7.56-7.57 (d, 1H, methoxyquinoline), 7.68-7.70 (d, 1H, methoxyquinoline), 8.62 (s, 1H, methoxyquinoline), 9.03-9.04 (d, 1H, methoxyquinoline). ^{13}C NMR (DMSO-d_6): 21.81, 56.19, 100.59, 108.14, 117.32, 118.46, 121.20, 125.29, 139.07, 140.62, 151.50, 151.58, 153.03, 155.18, 161.24, 168.03. LCMS: *m/z* 309.1 (M+1). Anal. calcd. for $C_{16}H_{12}N_4O_3$: C-62.33, H-3.92, N-18.17 %; Found C-62.30, H-3.94, N-18.18 %.

General procedure of 1-(7-methoxyquinolin-4-yl)-6-methyl-5-aryl-1,5-dihydro-4*H*-pyrazolo[3,4-d]pyrimidin-4-one (6a-e): To the compound 1-(7-methoxyquinolin-4-yl)-6-methylpyrazolo[3,4-d][1,3]oxazin-4(1*H*)-one (**5**) (1.0 g, 0.003 mol) taken in 10 mL phosphorus oxychloride was added 1 M equivalent of substituted aniline and heated to 100-105 °C for 8 h on an oil bath. It was then cooled to room temperature and poured into ice-cold water, neutralized with sodium bicarbonate solution, the solid filtered and washed with water. The crude product was recrystallized from ethyl acetate.

5-(5-Chloro-2-methylphenyl)-1-(7-methoxyquinolin-4-yl)-6-methyl-1,5-dihydro-4*H*-pyrazolo[3,4-d]pyrimidin-4-one (6a): ^{1}H NMR ($CDCl_3$): δ 1.16 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 6.80 (s, 1H, phenyl), 7.15-7.28 (m, 1H, phenyl), 7.39-7.43 (d, 1H, methoxyquinoline), 7.44-7.44 (d, 1H, pyrazole), 7.46-7.47 (d, 1H, methoxyquinoline), 7.85-7.87 (d, 1H, methoxyquinoline), 8.31 (s, 1H, methoxyquinoline), 8.89-8.92 (d, 1H, methoxyquinoline). ^{13}C NMR (DMSO-d_6): 23.99, 24.30, 55.64, 105.26, 107.40, 116.19, 117.08, 117.27, 118.78, 120.67, 120.86, 124.74, 124.87, 125.35, 125.45, 125.49, 129.99, 131.73, 131.81, 137.67, 141.28, 150.26, 151.76, 152.66, 156.33, 157.46, 158.82, 159.03, 161.16. LCMS: *m/z* 432.0 (M+1). Anal. calcd. for $C_{23}H_{18}N_5O_2Cl$: C-63.96 %, H-4.20 %, N-16.22 %; Found C-63.94 %, H-4.22 %, N-16.20 %.

5-(2-Bromophenyl)-1-(7-methoxyquinolin-4-yl)-6-methyl-1,5-dihydro-4*H*-pyrazolo[3,4-d]pyrimidin-4-one (6b): ^{1}H NMR ($CDCl_3$): δ 2.11 (s, 3H, CH_3), 3.93 (s, 3H, OCH_3), 7.19-7.37 (m, 1H, phenyl), 7.45-7.54 (d, 1H, methoxyquinoline), 7.56-7.73 (d, 1H, pyrazole), 7.75-7.75 (d, 1H, methoxyquinoline), 7.94-7.96 (d, 1H, methoxyquinoline), 8.3 (s, 1H, methoxyquinoline), 8.9 (d, 1H, methoxyquinoline).^{13}C NMR (DMSO-d_6): 24.16, 55.72, 105.50, 107.05, 115.98, 118.72, 121.04, 122.87, 125.58, 129.28, 129.82, 131.26, 134.14, 136.67, 137.87, 141.60, 149.77, 151.37, 152.74, 157.18, 158.89, 161.35. LCMS: *m/z* 463.9 (M+1). Anal. calcd. for $C_{22}H_{16}N_5O_2Br$: C-57.16, H-3.49, N-15.15 %; Found C-57.13, H-3.50, N-15.17 %.

5-(3-Chloro-4-methylphenyl)-1-(7-methoxyquinolin-4-yl)-6-methyl-1,5-dihydro-4*H*-pyrazolo[3,4-d]pyrimidin-4-one (6c): ^{1}H NMR ($CDCl_3$): δ 2.16 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 3.92 (s, 3H, OCH_3),7.10-7.11 (s, 1H, phenyl), 7.12-7.20 (m, 1H, phenyl), 7.42-7.44 (d, 1H, methoxyquinoline), 7.45-7.45 (d, 1H, pyrazole), 7.46-7.49 (d, 1H, methoxyquinoline), 7.85-7.87 (d, 1H, methoxyquinoline), 8.32 (s, 1H, methoxyquinoline), 8.91-8.92 (d, 1H, methoxyquinoline). ^{13}C NMR (DMSO-d_6): 20.55, 24.87, 55.65, 105.21, 107.58, 116.24, 118.79, 120.91, 124.86, 125.15, 132.81, 134.63, 135.66, 135.90, 137.62, 141.09, 149.77, 150.42, 151.93, 152.42, 167.89, 158.23, 161.16. LCMS: *m/z* =432.0 (M+1). Anal. calcd. for $C_{23}H_{18}N_5O_2Cl$: C-63.96 %, H-4.20 %, N-16.22 %; Found C-63.93, H-4.20 %, N-16.24 %.

5-(4-Bromo-2-fluorophenyl)-1-(7-methoxyquinolin-4-yl)-6-methyl-1,5-dihydro-4*H*-pyrazolo[3,4-d]pyrimidin-4-one (6d): ^{1}H NMR ($CDCl_3$): δ 2.15 (s, 3H, CH_3), 3.93 (s, 3H, OCH_3),7.17-7.20 (m, 1H, phenyl), 7.36-7.39 (d, 1H, methoxyquinoline), 7.43 (s, 1H, phenyl), 7.44-7.47 (d, 1H, methoxyquinoline), 7.50-7.52 (d, 1H, pyrazole), 7.80-9.90 (d, 1H, methoxyquinoline), 8.3 (s, 1H, methoxyquinoline), 8.92-8.93 (d, 1H, methoxyquinoline). ^{13}C NMR (DMSO-d_6): 23.98, 55.69, 105.15, 107.22, 116.15, 118.76, 120.91, 121.03, 121.13, 123.94, 124.08, 124.48, 124.57, 125.33, 128.96, 129.00, 131.06, 137.73, 141.36, 150.03, 151.56, 152.58, 156.14, 157.21, 158.60, 158.69, 161.29. LCMS: *m/z* = 479.9 (M+1), 481.9 (M+2). Anal. calcd. for $C_{22}H_{15}N_5O_2BrF$: C-55.02, H-3.15, N-14.58 %; Found:C-55.04, H-3.16, N-14.56 %.

1-(7-Methoxyquinolin-4-yl)-6-methyl-5-(3-methylphenyl)-1,5-dihydro-4*H*-pyrazolo[3,4-d]pyrimidin-4-one (6e): ^{1}H NMR ($CDCl_3$): δ 2.04 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 3.92 (s, 3H, OCH_3),7.13 (s, 1H, phenyl), 7.13-7.20 (m, 1H, phenyl), 7.27-7.29 (d, 1H, methoxyquinoline), 7.33-7.33 (d, 1H, pyrazole), 7.35-7.35 (d, 1H, methoxyquinoline), 7.89-7.92 (d, 1H, methoxyquinoline), 8.33 (s, 1H, methoxyquinoline), 8.91-8.92 (d, 1H, methoxyquinoline). ^{13}C NMR (DMSO-d_6): 16.98, 24.25, 55.69, 105.41, 107.25, 116.03, 118.73, 120.98, 125.42, 128.14, 130.13, 132.68, 132.97, 134.12, 137.30, 137.71, 141.41, 150.01, 151.60, 152.70, 157.25, 158.74, 161.28. LCMS: *m/z* 398.3 (M+1). Anal. calcd. for $C_{23}H_{19}N_5O_2F$: C-69.51, H-4.82, N-17.62 %; Found:C-69.50, H-4.80, N-17.64 %.

Molecular docking using HEX 8.0: It has become increasingly clear that impaired deactivation of RTKs may be a mechanism in cancer [26]. Further, normal cancer cells have receptors that attach to circulating estrogen and progesterone. Estrogen and progesterone bind to the receptors that may work with growth factors (*e.g.*, oncogenes and mutated tumor suppressor genes) to cause cancer cell growth [27]. Based on the literature it has been shown clearly that the drug toremifene has been used to target the human estrogen receptor [28]. Bearing above facts, we selected human estrogen receptor and RTKs as a biological targets and human estrogen receptor (PDB ID: 2IOK) and the crystal structure of EGFR kinase domain (PDB ID: 2a91) were retrieved from protein data bank for docking study of synthesized compounds using HEX 8.0 software.

For macromolecular docking studies, the chemical structures of synthesized ligand, metal complexes and standard toremifene were drawn using ChemDraw ultra. The 3D optimization was done in ChemDraw 3D ultra software and stored as pdb file. Hex docking was carried out by setting suitable parameters (Table-1). This docking score can be interpreted as interaction energy. More negative E – Total value implies that there exists a strong interaction between drug and receptor that leads to inhibition of receptor activity.

RESULTS AND DISCUSSION

The starting compound 4-chloro-7-methoxy quinolone (**1**) condensed with hydrazine hydrate in alcoholic medium, smoothly underwent nucleophilic substitution to give 4-hydrazino-7-methoxy quinolone (**2**) in good yield. The key intermediate 4-hydrazino-7-methoxy quinoline condensed with ethoxymethylenecyanoacetate afforded ethyl 5-amino-1-(7-methoxyquinolin-4-yl)-1*H*-pyrazole-4-carboxylate (**3**). The 400 MHz ^{1}H NMR of compound **3** showed the signals at δ 1.29 triplet (3H) and 4.2 quartet (2H) indicating the presence of an ethyl group of the ester, δ 6.7 singlet corresponding to two protons indicating the presence of NH_2 group in the pyrazole and δ 7.53 singlet, which corresponds to the pyrazole ring proton and δ 4.0 triplet (3H) indicates for methoxyquinolinering proton. Compounds **3** upon basic hydrolysis gave compound 5-amino-1-(7-methoxyquinolin-4-yl)-1*H*-pyrazole-4-carboxylic acid (**4**) in excellent yield. The spectral data revealed the formation of the compound 5-amino-1-(7-methoxyquinolin-4-yl)-1*H*-pyrazole-4-carboxylic acid (**4**). The compound **4**, when heated with acetic anhydride affords cyclized product1-(7-methoxyquinolin-4-yl)-6-methylpyrazolo[3,4-d][1,3]oxazin-4(1*H*)-one (**5**). ^{1}H NMR spectrum revealed the

disappearance of both NH_2 and OH peaks and the appearance of a singlet at δ 2.39, which corresponds to methyl protons, confirming the formation of the compound (**5**). Further, the compound **5** was treated with substituted aromatic amines in the presence of phosphorus oxychloride to yield the compounds **6(a-e)** (**Scheme-I**). The newly synthesized compounds were analyzed by elemental analysis and spectral data. The spectral data of all these compounds supported the assigned structures. The physical data of the synthesized compounds is tabulated in Table-1.

TABLE-1
PHYSICAL DATA OF COMPOUNDS **4** TO **6(a-e)**

Compd.	m.f.	m.w.	m.p. (°C)	Yield (%)
3	$C_{16}H_{16}N_4O_3$	312.32	167-168	65
4	$C_{14}H_{12}N_4O_3$	284.27	201-203	80
5	$C_{16}H_{12}N_4O_3$	308.29	151-153	68
6a	$C_{23}H_{18}N_5O_2Cl$	431.87	212-214	65
6b	$C_{22}H_{16}N_5O_2Br$	462.69	196-196	60
6c	$C_{23}H_{18}N_5O_2Cl$	431.87	218-220	55
6d	$C_{22}H_{15}N_5O_2BrF$	480.28	230-233	58
6e	$C_{23}H_{19}N_5O_2F$	397.42	225-227	60

Biological activity

Antibactrial activity: The antibacterial activity of the compounds was tested against Gram-positive bacteria, namely, *Staphylococcus aureus, Bacillus cereus* and against Gram-negative bacteria, namely, *Pseudomonas aeruginosa, Klebsiella pneumonia, Vibrio cholera, Shigella flexneri* and *Escherichia coli* by agar well diffusion method [29]. The 24 h old Muller-Hinton broth cultures of test bacteria were swabbed on sterile Muller-Hinton agar plates using sterile cotton swab followed by punching wells of 6 mm with the help of sterile cork borer. The standard drug (chloramphenicol, 1 mg/mL of sterile distilled water), compounds **6(a-e)** (20 mg/mL of 10 % DMSO) and control (10 % DMSO) were added to, respectively, labeled wells. The plates were allowed to stand for 0.5 h and incubated at 37 °C for 24 h and the zone of inhibition was recorded.The results of such studies are given in Table-2.

TABLE-2
ANTIBACTERIAL ACTIVITY OF COMPOUNDS **6(a-e)**

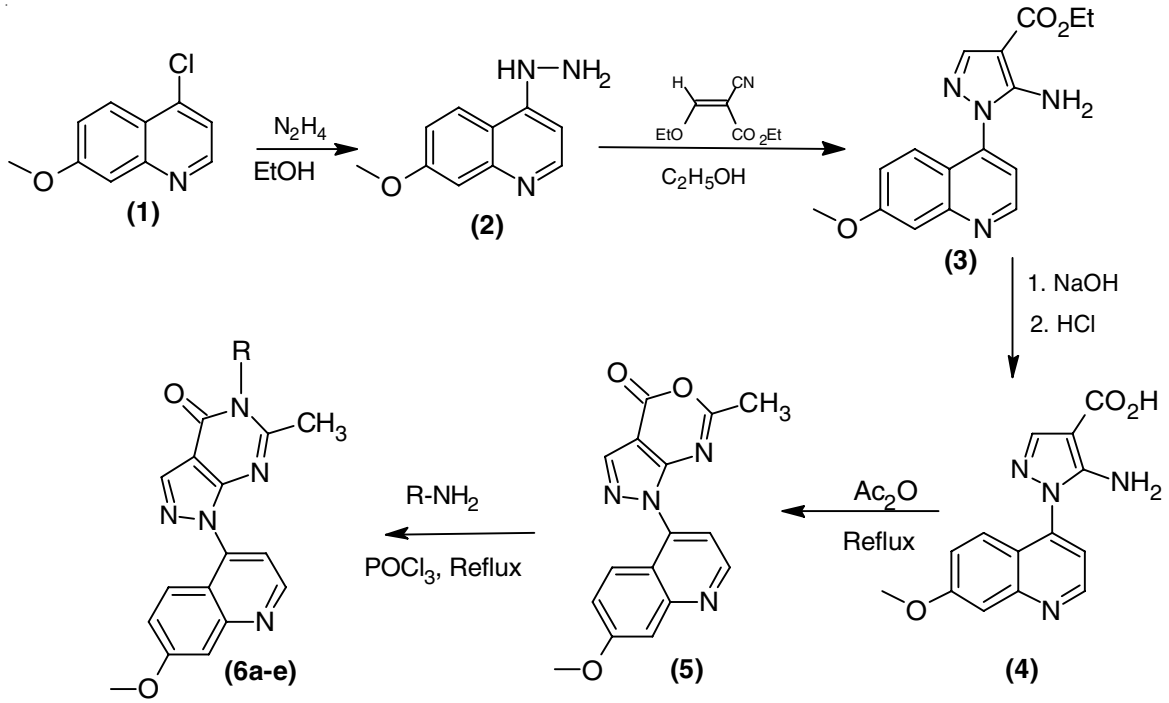
Compd.	Zone of inhibition at 20 mg/mL (mm)						
	VC	SA	KP	PA	BC	EC	SF
6a	2.0	2.1	1.9	1.9	2.2	1.8	2.0
6b	2.4	2.6	2.3	2.3	2.7	2.3	2.5
6c	2.5	2.7	2.5	2.5	2.9	2.5	2.7
6d	1.9	2.0	1.8	1.6	1.7	1.6	1.7
6e	2.2	2.4	2.0	2.0	2.5	2.1	2.2
Standard	2.8	3.0	2.7	2.8	3.1	2.8	2.9

VC = *V. cholerae*; SA = *S. aureus*; KP = *K. pneumoniae*;
PA = *P. aeruginosa*; BC = *B. cereus*; EC = *E. coli*; SF = *S. flexneri*

The data showed that compound **6c**, **6b** and **6e** showed potent antibacterial activity and remaining compounds were found to have moderate activity against the tested organisms.

Antioxidant studies

DPPH assay: The radical scavenging ability of synthesized compounds and the ascorbic acid (standard) was tested on the basis of radical scavenging effect on 2,2-diphenyl-1-picryhydrazyl radical DPPH free radical. Different concentrations (5, 10, 25, 50, 100 mg/mL) of compounds and standard were prepared in methanol. In clean and labeled test tubes, 2 mL of DPPH solution (0.002 % in methanol) was mixed with 2 mL of different concentrations of compounds and standard separately. The tubes were incubated at room temperature in



R = **6a**: 5-chloro-2-methyl; **6b**: 2-bromo; **6c**: 3-chloro-4-methyl; **6d**: 4-bromo-2-fluoro; **6e**: 3-methyl

Scheme-I: Synthetic route of compounds **6(a-e)**

dark for 0.5 h and optical density was measured at 517 nm using UV-visible spectrophotometer. The absorbance of the DPPH control was also noted. The scavenging activity was calculated using the formula: scavenging activity (%) = A-B/A × 100, where A is the absorbance of DPPH and B is the absorbance of DPPH in standard combination [30]. The compounds **6c** and **6b** exhibited prolific DPPH scavenging activity followed by the compounds **6e**, **6a** and **6d**. The results are summarized in Table-3.

TABLE-3
DPPH RADICAL SCAVENGING ACTIVITY OF COMPOUNDS **6(a-e)**

Compounds	Scavenging activity of different concentrations (µg/mL) in %				
	5	10	25	50	100
6a	41.25	44.28	53.16	61.65	68.22
6b	49.50	53.53	58.34	63.46	70.65
6c	53.58	56.34	62.67	65.67	71.34
6d	39.10	41.45	50.32	58.55	66.54
6e	45.46	48.12	55.45	62.12	70.13
Ascorbic acid	85.90	90.56	93.50	95.65	98.90

Ferric reducing assay: Different concentrations (5, 10, 25, 50, 100 mg/mL) of compounds and standard (tannic acid) in 1 mL methanol were mixed in separate tubes with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of 1 % potassium ferricyanide. The tubes were placed in water bath for 20 min at 50 °C, cooled rapidly and mixed with 2.5 mL of 10 % trichloroacetic acid and 0.5 mL of 0.1 % ferric chloride. The amount of iron(II)-ferricyanide complex formed was determined by measuring the formation of Perl's Prussian blue at 700 nm after 10 min. The increase in absorbance of the reaction mixture indicated the increased reducing power [31]. The compounds **6b** and **6d** exposed fruitful radical scavenging activity. The results are summarized in Table-4.

TABLE-4
FERRIC REDUCING ACTIVITY OF COMPOUNDS **6(a-e)**

Compounds	Radical scavenging activity of different concentrations (µg/mL) in %				
	5	10	25	50	100
6a	0.39	0.41	0.48	0.53	0.57
6b	0.41	0.45	0.52	0.56	0.61
6c	0.37	0.39	0.45	0.51	0.55
6d	0.40	0.43	0.50	0.55	0.59
6e	0.36	0.40	0.43	0.49	0.53
Tannic acid	0.45	0.50	0.56	0.62	0.69

***in silico* Molecular docking studies:** For human estrogen receptor, all compounds exhibited more binding interaction energy against the receptor with least docking score compared to the ligand and standard and hence complexes may be considered as potential inhibitors of human estrogen receptor. Similarly for tyrosine kinase, docking score obtained for synthesized compounds are comparable with the standard. Among all compounds docked (Fig. 1) compound **6c** showed comparatively least E-total value -171.05 kJ mol^{-1} is significantly having more inhibiting ability towards tyrosine kinase receptor, which binds to the active site of the receptors thereby potentially inhibits the cancer causing property of the receptor. Form Table-5 it can be concluded that all compounds potentially inhibit the human estrogen receptor and tyrosine kinase (RTK) and especially, compound **6c** was found to be potential inhibitor of both the receptors.

Figs. 1-6 are docking pictures of synthesized compound with ciprofloxacin.

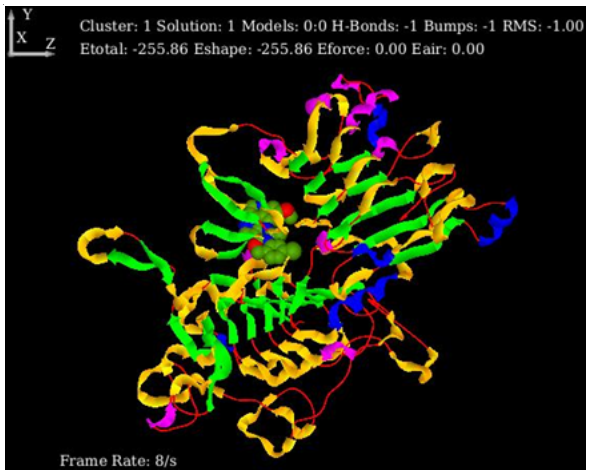


Fig. 1

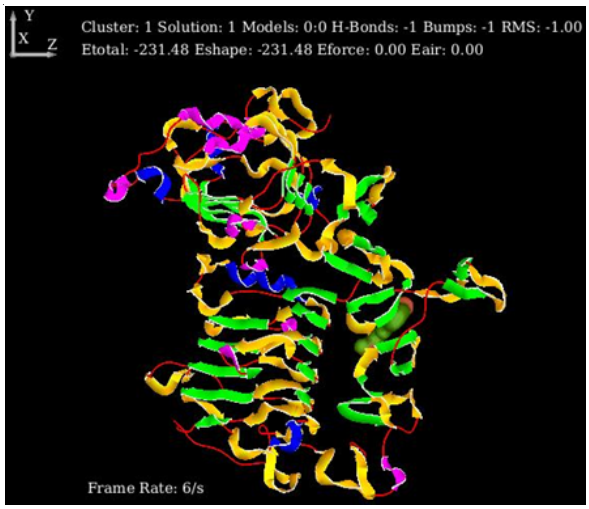


Fig. 2

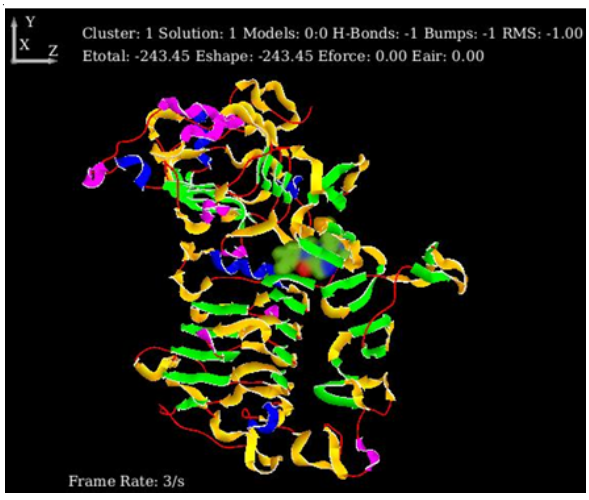


Fig. 3

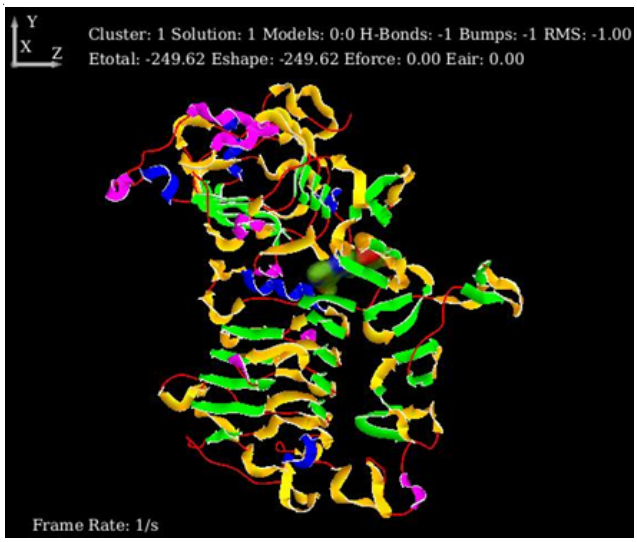


Fig. 4

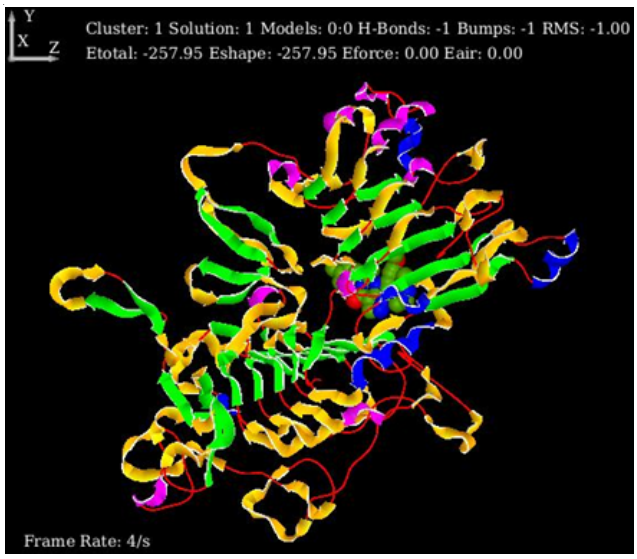


Fig. 5

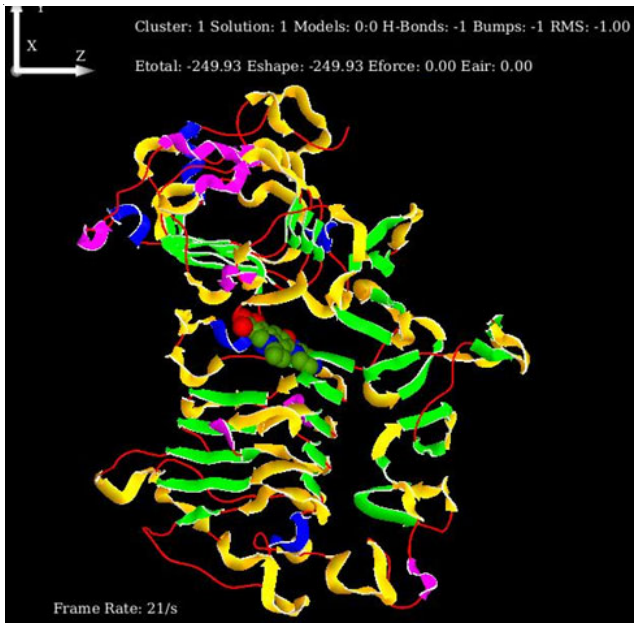


Fig. 6

Conclusion

A series of novel 1-(7-methoxyquinolin-4-yl)-6-methyl-5-aryl-1,5-dihydro-4*H*-pyrazolo[3,4-d]pyrimidin-4-one derivatives **6(a-e)** have been synthesized through a facile strategy and characterized by ^{1}H NMR, ^{13}C NMR, mass spectral data. The target molecules have been screened for antibacterial and antioxidant activities. Compounds **6c**, **6b** and **6d** showed promising antibacterial and DPPH scavenging activities while the compounds **6b** and **6d** emerged as potent radical scavengers. In molecular docking studies, compound **6c** showed minimum binding energy. The compounds with minimum binding energy are responsible for more active antimicrobial agent with respect to standard drugs. The best dock conformation is one; with least binding energy has the highest affinity.

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