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Novel series of benzo[*d*]thiazolyl substituted-2-quinolone hybrids: Design, synthesis, biological evaluation and *in-silico* insights

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ABSTRACT

A novel series of 3-(2-(4-(substituted-benzo[*d*]thiazol-2-yl)phenylamino)acetyl)-4–hydroxy-1-methyl/phenyl quinolin-2(1H)-one (**7a-f** and **8a-f**) were synthesized. Reaction of appropriately substituted-2-(4-amino phenyl)benzo[*d*]thiazole (**4a-f**) with 3-(2-bromoacetyl)-4–hydroxy-1-methyl/phenyl quinolin-2(1H)-one (**5**/**6**) in the presence of glacial acetic acid resulted in desired compounds. Structures of the synthesized compounds were characterized based on their spectral (IR, ^{1}H NMR, ^{13}C NMR and MS) and elemental analysis. The cytotoxicity screening studies revealed that MCF-7 and WRL68 cancer cells were sensitive to all the tested compounds. Out of twelve novel hybrids, compound **8f** displayed the most significant anticancer activity. Docking studies were performed in order to understand the binding mode of the title compounds at the active site of the target enzyme (EGFR tyrosine kinase, **1M17**). Compounds **8f** and **7f** displayed prominent and conserved binding interactions against **1M17**. In addition, compounds **7e, 7f, 8e,** and **8f** exhibited interesting *in vitro* antibacterial activity, especially against Gram-negative bacteria *E. coli*. In summary, the novel benzo[*d*]thiazolyl substituted-2-quinolone hybrid (**8f**) could be considered as promising hit and could be further exploited for developing potential anticancer/antimicrobial agents.

1. Introduction

A grave health problem across the globe 'Cancer' is a chronic pathological condition, where there is a rapid uncontrolled proliferation of abnormal premature cells. Cancer may affect people at all ages, even fetuses, but risk tends to increase with age [1]. The active cases of cancer are increasing at an alarming rate due to drastic change in lifestyle of humans over the years. It is the second most common cause of death after heart diseases. Approximately 21% annual deaths worldwide occur due to cancer *i.e.* 7.6 million deaths annually and is expected to reach up to 13 million in 2030 [2]. Despite increase in sophisticated approach in designing cancer chemotherapy, there is still no curative treatment that is 100% effective. Currently cancer chemotherapeutic agents lack selectivity and specificity against cancerous cells/tissues, which is a significant setback. Consequently, cancer chemotherapy targeting tumor represents one of the most significant challenges for chemists and oncologists [3].

Heterocyclic skeletons serve as ideal scaffolds on which pharmacophores can be appended to yield potent and selective drugs [4]. In the past two decades, 2-substituted benzo[*d*]thiazole analogues have been extensively studied for their anticancer activity and continued to magnetize considerable attention in anticancer research [5–8]. Literature reports suggest that 2-(4-Amino phenyl)benzo[*d*]thiazoles [9] and their corresponding N-acetylated derivatives [10] possess remarkable *in vitro* anticancer activity especially against breast, colon, and ovarian cell lines. Fig. 1 illustrates the importance of benzo[*d*]thiazole nucleus and its analogs with their potential antitumor activities [11–15]. This versatile and potent scaffold has provided an impetus for the anticancer drug discovery in recent years.

Another important class of heterocyclic scaffold is 2-quinolone (also known as carbostyril or 1-aza coumarin), which serves as a privileged structure for the generation of drug-like libraries in drug-discovery programs. In addition, 2-quinolones are iso-

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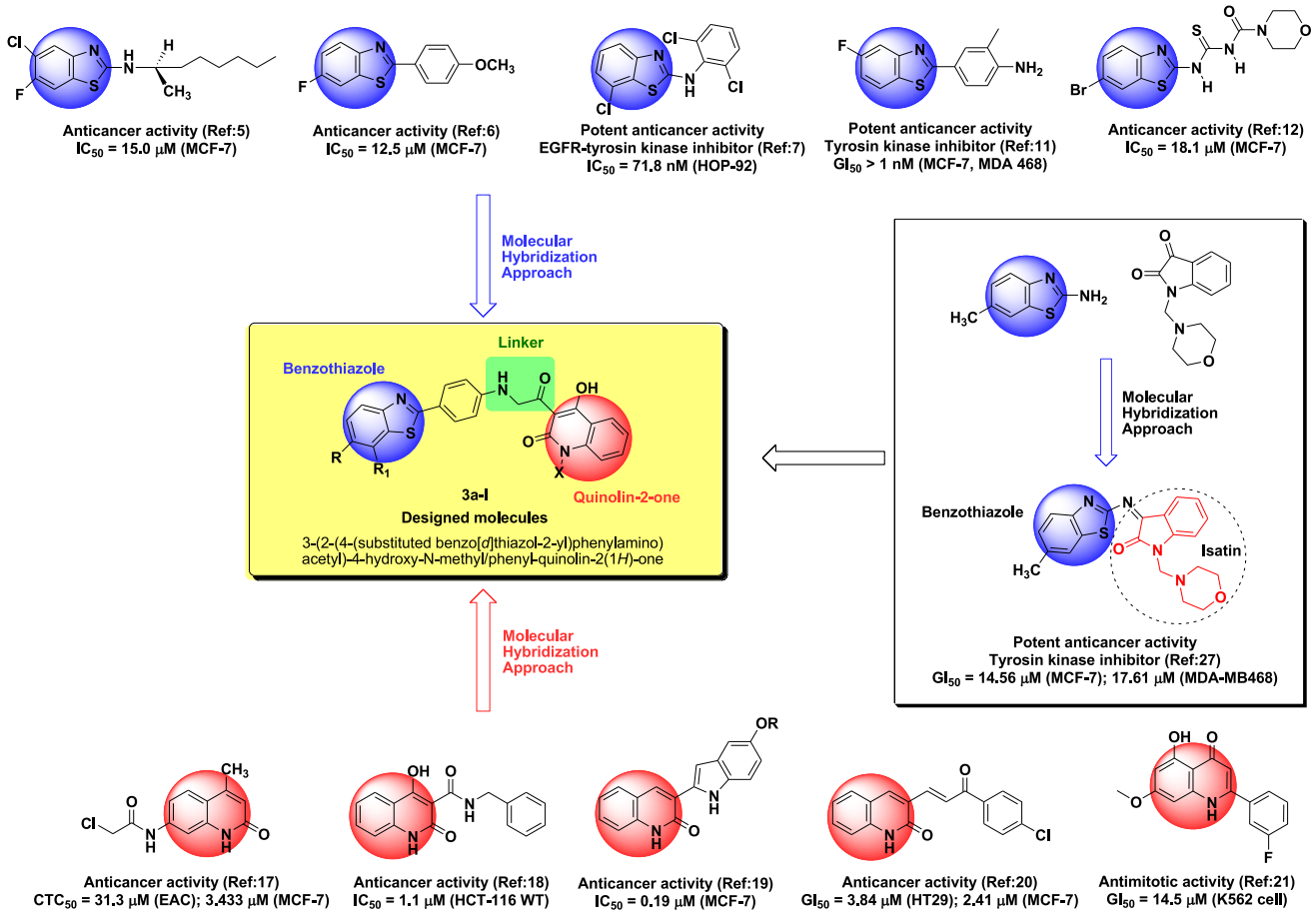


Fig. 1. Reported and Proposed structures of anticancer benzo[*d*]thiazole and 2-quinolone derivatives. Design of a novel series of 2-phenylbenzo[*d*]thiazolyl substituted 2-quinolone analogs using molecular hybridization approach.

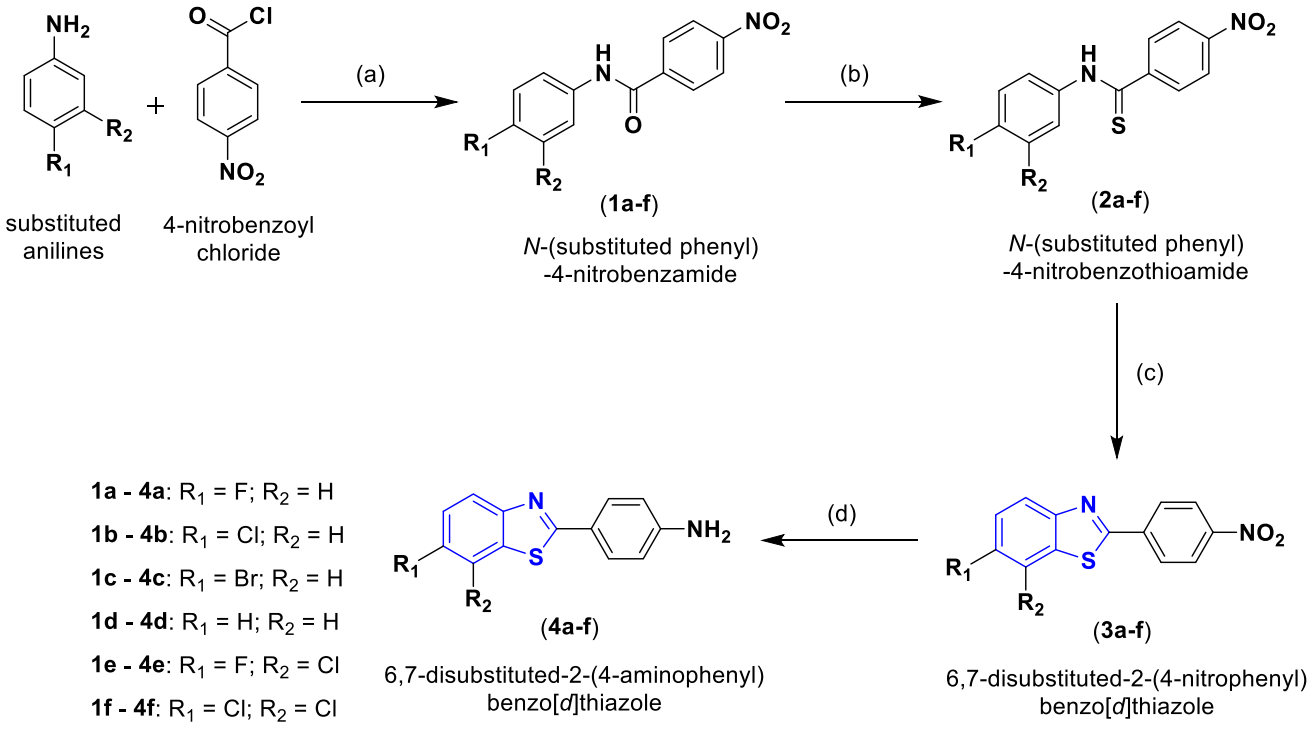
meric to 4-quinolones and isosteric to coumarins and are associated with interesting biologic activities such as antibacterial, anticancer, antiviral, cardiotonic, etc. [16–21]. During the last decade, 2-quinolones have been extensively studied for their anticancer activity as represented in Fig. 1. Further, Ruiz et al. reported cytotoxic mechanism of quinolone derivatives as alkylating agents [22]. Different substituted 2-quinolones have also show *in-vitro* and *in vivo* growth inhibition activity against various strains of bacteria and fungi; moreover quinolones are also known to inhibit DNA synthesis by promoting cleavage of bacterial DNA gyrase and type-IV topoisomerase, resulting in rapid cell death [23–25].

Over the years, molecular hybridization based drug design approach [26,27] has been exploited by many researchers in order to develop some promising new hybrid chemical entities (NHCEs), displaying significant therapeutic values. The combination of two pharmacophores into a single molecular skeleton is a well established approach for designing more potent drugs with significant increase in activity. A hybrid molecule acting on manifold targets is considered to be a better drug candidate than drug combinations, since administration of single drug will have more predictable pharmacokinetic and pharmacodynamic properties with improved patient compliance [28]. Owing to their well appreciated anticancer and antimicrobial properties, it was envisaged to design hybrid structures having substituted 2-phenylbenzo[*d*]thiazole and substituted 2-quinolone motifs connected with a linker (Fig. 1). Therefore, in view of the above facts and in continuation of search on biologically active hybrid molecules [29–33], in this report the synthesis of novel benzo[*d*]thiazolyl-2-quinolone hybrid analogs (**7a-f** and **8a-f**) with their subsequent *in vitro* biological evaluation for anticancer and antimicrobial activities is communicated.

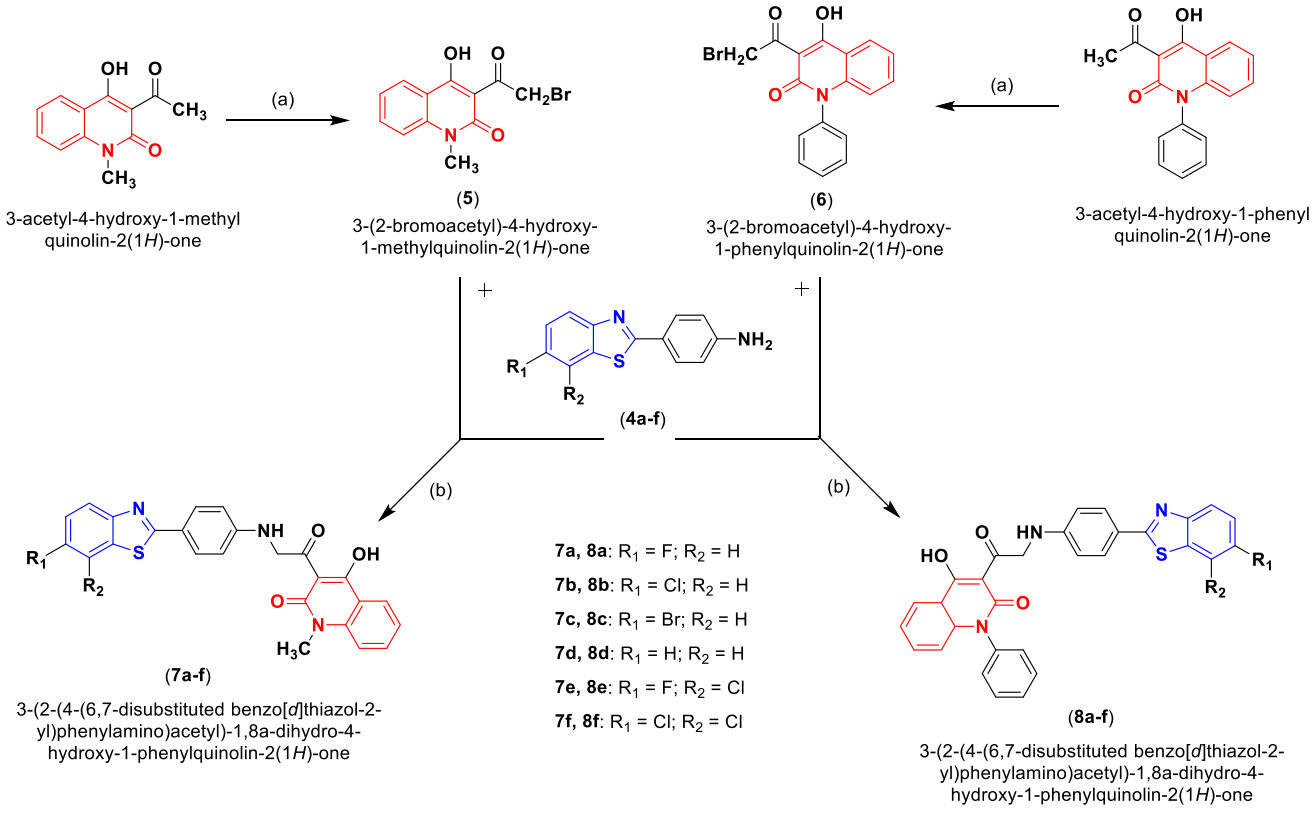
2. Results and discussion

2.1. Synthetic and spectral studies

The synthesis of series of twelve novel 3-(2-(4-(6,7-substituted-benzo[*d*]thiazol-2-yl)phenylamino)acetyl)−4−hydroxy-1-methyl/phenyl quinolin-2(1H)-one (**7a-f** and **8a-f**) derivatives was achieved through convenient and efficient synthetic route as outlined in Schemes 1 and 2. The cyclization of N-(3,4-disubstituted-phenyl)−4-nitrobenzothioamide with potassium ferricynide in presence of aqueous sodium hydroxide afforded 6,7-substituted-2-(4-nitrophenyl)benzo[*d*]thiazole, which upon reduction with stannous chloride yielded the key intermediate compounds *i.e.* 6,7-disubstituted-2-(4-aminophenyl)benzo[*d*]thiazole (**4a-f**) following the Jacobson synthetic method [34] as depicted in Scheme 1. The structures of these intermediates were characterized by IR and NMR spectral studies (Table 1). The IR spectrum of compound **4a** showed two characteristic sharp absorption bands at 3425.63 cm^{-1} and 3333.24 cm^{-1}, which was attributed to the amino (-NH_2) group at *para* position of phenyl ring of 2-phenylbenzo[*d*]thiazole moiety, while the absorption band appeared at 1654.11 cm^{-1} due to $C = N$ bond confirmed the formation of benzo[*d*]thiazole ring. This is further substantiated from ^{1}H NMR spectrum of compound **4a**, which showed presence of characteristic singlet peak at δ 7.87 ppm attributed for amino (NH_2) protons, while the aromatic protons appeared as multiplets

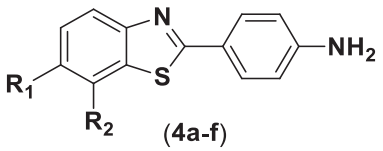


Scheme 1. Synthetic outline of key intermediates 6,7-disubstituted-2-(4-aminophenyl)benzo[*d*]thiazole (**4a-f**). *__Reagents and Conditions__: (a) Pyridine, reflux, 2–5 h; (b) Lawesson's reagent, HMPA, 100 °C, stir, 6–9 h; (c) 10% Aqueous NaOH, Potassium ferricynide, 90 °C, stir, 1–2 h; (d) Tin(II)chloride dehydrate, Absolute ethanol, nitrogen environment, stir, 3–5 h.



Scheme 2. Synthetic route of 3-(2-bromoacetyl)−4-hydroxy-1-methyl/phenyl quinolin-2(1*H*)-one (**5** and **6**) and the corresponding benzo[*d*]thiazolyl disubstituted-2-quinolone hybrid analogs (**7a-f** and **8a-f**). *__Reagents and Conditions__: (a) Bromine, Glacial acetic acid, Δ, 80 °C, stir; (b) Glacial acetic acid, stir, reflux, 6–13 h.

Table 1
Physicochemical and spectral characterization data of 6,7-disubstituted-2-(4-aminophenyl)benzo[*d*]thiazole (**4a-f**).



(4a-f)

Comp	R_1	R_2	Mol formula	MP °C	Rf*	FTIR (KBr disk, Cm^{-1})	^1H NMR (DMSO–d_6 δ, ppm)
4a	F	H	$C_{13}H_9FN_2S$	171–173	0.25	3425.63, 3420.24 (NH_2 Str), 3125.35 (C–H Str), 1654.11 ($C = N$)	6.67–7.38 (m, 6H, Ar-H), 7.87 (s, 2H, NH_2), 7.97 (m, 1H, Ar-H)
4b	Cl	H	$C_{13}H_9ClN_2S$	194–196	0.64	3498.74, 3489.29 (NH_2 Str), 3077.67 (C–H Str), 1674.80 ($C = N$)	6.81–7.04 (m, 5H, Ar-H), 7.20–7.29 (m, 1H, Ar-H), 7.40 (s, 2H, NH_2), 7.91 (m, 1H, Ar-H)
4c	Br	H	$C_{13}H_9BrN_2S$	161–163	0.34	3469.06, 3450.57 (NH_2 Str), 3078.69 (C–H Str), 1662.20 ($C = N$)	6.71–7.30 (m, 5H, Ar-H), 7.35 (s, 2H, NH_2), 7.60–7.65 (m, 1H, Ar-H)
4d	H	H	$C_{13}H_{10}N_2S$	267–269	0.79	3454.17, 3433.57 (NH_2 Str), 3080.10 (C–H Str), 1659.11 ($C = N$)	7.01–8.08 (m, 8H, Ar-H), 7.57 (s, 2H, NH_2)
4e	F	Cl	$C_{13}H_8ClFN_2S$	180–182	0.59	3433.45, 3426.20 (NH Str), 3083.46 (C–H Str), 1648.10 ($C = N$)	6.93–7.18 (m, 5H, Ar-H), 7.16–7.22 (m, 1H, Ar-H), 7.91 (s, 2H, NH_2)
4f	Cl	Cl	$C_{13}H_8Cl_2N_2S$	189–191	0.91	3477.73, 3462.41 (NH_2 Str), 3101.12 (C–H Str), 1653.47 ($C = N$)	7.04–7.77 (s, 5H, Ar-H), 7.85 (s, 2H, NH_2), 8.02 (m, 1H, Ar-H)

between δ 6.67–7.97 ppm, indicating its formation by simple cyclization process.

Further, compounds 3-(2-bromoacetyl)−4–hydroxy-1-methyl/phenyl quinolin-2(1*H*)-one (**5** and **6**) were prepared [35], which involves the reaction of 3-acetyl-4–hydroxy-1-methyl/phenyl quinolin-2 (1*H*)-one with the calculated quantities of bromine in the presence of glacial acetic acid as shown in Scheme 2. The spectral details of compounds **5** and **6** are summarized in experimental section. The IR spectrum of **5** showed a characteristic broad band at 3469.02 cm^{-1} due to the hydroxyl (OH) group, while the most prominent peaks appeared at 1737.08 cm^{-1} and 1658.22 cm^{-1} attributed to the carbonyl groups ($C = O$) of acetyl (-$COCH_2Br$) and amide (-CONH of 2-quinolone), respectively. This is further evidenced from the ^1H NMR spectrum of compound **5**, which showed a characteristic singlet at δ 3.23 ppm indicating the presence of acetyl (-COC $\underline{\mathbf{H}}_2$Br) protons, while the *N*–CH_3 protons appeared as singlet δ 3.64 ppm and the aromatic protons resonated as multiplets between δ 7.22–8.68 ppm. From ^{13}C NMR spectrum of compound **5**, it was further confirmed that the most characteristic signals appeared at δ 33.45 and 201.03 ppm due to *N*-methyl (*N*–CH_3) carbon and acetyl (-$COCH_2$) carbon, respectively.

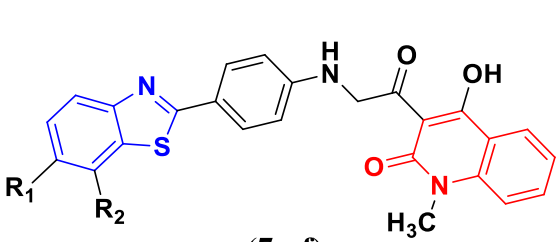
Further, the desired compounds 3-(2-(4-(substituted-benzo[*d*]thiazol-2-yl)phenyl amino)acetyl)−4–hydroxy-1-methyl/phenyl quinolin-2(1H)-one (**7a-f** and **8a-f**) were obtained by the reaction of 6,7-disubstituted-2-(4-aminophenyl)benzo[*d*]thiazole (**4a-f**) with 3-(2–bromo acetyl)−4–hydroxy-1-methyl/phenylquinolin-2(1H)-one (**5** or **6**) in presence of glacial acetic acid as illustrated in Scheme 2. Structures of final hybrid derivatives (**7a-f** and **8a-f**) were characterized based on their physicochemical, spectral (IR, ^1H NMR, ^{13}C NMR and MS), and elemental analysis. All the newly synthesized compounds showed acceptable analysis of their anticipated structures, which are summarized in Tables 2 and 3. The IR spectra of the title compounds (**7a-f** and **8a-f**) showed moderately strong bands around 3426.38–3498.74 cm^{-1}, 3335.23–3385.07 cm^{-1}, 3071.11–3089.96 cm^{-1}, and 1630.26–1639.87 cm^{-1}, which are characteristics of the O–H, N–H, C–H, and $C = N$ groups, respectively. The most informative and sharp bands appeared at 1716.03–1728.13 cm^{-1} and 1660.22–1672.90 cm^{-1} confirmed the presence of two distinctive carbonyl groups ($C = O$) of acetyl (-$COCH_2Br$) and amide (-$COCH_2NH$ of 2-quinolone), respectively. In ^1H NMR spectra, the characteristic O–H proton (singlet) of 2-quinolone ring appears at δ 10.39–11.45 ppm and the two distinctive singlets of N–H protons and methylene (–C $\underline{\mathbf{H}}_2$–CO) resonating at δ 8.50–8.74 and 4.87–5.64 ppm, respectively. Aromatic protons appeared as multiplets between δ 6.58–8.18 ppm while in the case of compounds **7a-f**, the *N*–C $\underline{\mathbf{H}}_3$ proton signals were observed around δ 3.15–3.62 ppm, respectively. Further, the formations of desired hybrid compounds were confirmed by recording their mass spectra, which were in full agreement with their molecular weights.

2.2. Anticancer activity

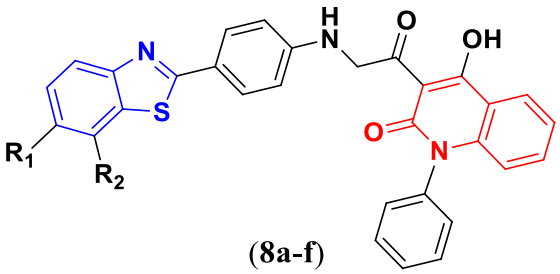
Anticancer drug development has become an extremely competitive and expensive process with high rate of failures. Generally, anticancer activities of most of the chemotherapeutic agents are due to their cytotoxicity. Anticancer activity of newly synthesized compounds (**7a-f** and **8a-f**) was assessed by MTT bioassay [36] and their respective cytotoxicity data (IC_{50} values) are summarized in Table 4. The title compounds were evaluated for their *in vitro* cytotoxic activity against four human cancer cell lines namely Ehrlich Ascites Carcinoma cells (**EAC**), hormone dependant breast cancer cells (**MCF7**), human colon carcinoma cells (**HT29**), and hepatic cancer cells (**WRL68**). Doxorubicin was taken as reference drug (positive control) in the study. These synthesized compounds exhibited an interesting anticancer activity profile against tested panel of cell lines and a brief structure activity relationship is discussed below.

EAC were found to be sensitive towards two of the twelve synthesized compounds (**7a-f** and **8a-f**). Among them, compounds **8f** (IC_{50} = 6.98, 4.44, 1.817 µM) and **7f** (IC_{50} = 9.34, 5.40, 3.08 µM) exhibited highest anticancer activity as compared to Doxorubicin (IC_{50} = 4.10, 1.86, 0.843 µM) against EAC at different time points of drug exposure. In the case of hormone dependant breast cancer cells, four compounds were found to be active out of twelve compounds tested. In particular, compound **8f** (IC_{50} = 2.22, 0.954 and 0.398 µM) displayed the notable anticancer activity while compounds **7e** (IC_{50} = 5.06, 3.33 and 1.76 µM), **7f** (IC_{50} = 10.51, 5.78 and 2.97 µM) and **8e** (IC_{50} = 11.09, 7.08 and 5.55 µM) displayed moderate to good activity against MCF7 at 24 h, 48 h and 72 h, respectively. Compound **8f** exhibited almost equipotent activity when compared to reference drug Doxorubicin (IC_{50} = 1.60, 0.625 and 0.259 µM). Further compound **8f** (IC_{50} = 10.21, 7.20 and 3.01 µM) also displayed most significant anticancer activity against HT29 cancer cells at different time intervals of drug exposure. This was the only compound from the series to exhibit potent anticancer

Table 2
Physicochemical and elemental analysis data of a novel series of 3-(2-(4-(6,7-disubstituted-benzo[*d*]thiazol-2-yl)phenylamino)acetyl)−4-hydroxy-1-methyl/phenyl quinolin-2(1H)-one derivatives.



(7a-f)



(8a-f)

Comp	R_1	R_2	Molecular formula	MP (°C)	Rf*	Elemental (CHN) Analysis:% Found (Calcd)		
						C	H	N
7a	F	H	$C_{25}H_{18}FN_3O_3S$	194–196	0.57	65.34 (65.34)	3.92 (3.95)	9.16 (9.14)
7b	Cl	H	$C_{25}H_{18}ClN_3O_3S$	176–178	0.53	63.03 (63.09)	3.79 (3.81)	8.89 (8.83)
7c	Br	H	$C_{25}H_{18}BrN_3O_3S$	209–211	0.47	57.68 (57.70)	3.43 (3.49)	8.10 (8.07)
7d	H	H	$C_{25}H_{19}N_3O_3S$	154–156	0.31	67.99 (68.01)	4.35 (4.34)	9.51 (9.52)
7e	F	Cl	$C_{25}H_{17}ClFN_3O_3S$	203–205	0.41	60.78 (60.79)	3.44 (3.47)	7.16 (7.18)
7f	Cl	Cl	$C_{25}H_{17}Cl_2N_3O_3S$	245–247	0.77	58.86 (58.83)	3.33 (3.36)	8.21 (8.23)
8a	F	H	$C_{30}H_{20}FN_3O_3S$	222–224	0.39	69.11 (69.09)	3.89 (3.87)	8.04 (8.06)
8b	Cl	H	$C_{30}H_{20}ClN_3O_3S$	199–201	0.68	67.00 (66.97)	3.76 (3.75)	7.79 (7.81)
8c	Br	H	$C_{30}H_{20}BrN_3O_3S$	265–267	0.36	61.89 (61.86)	3.44 (3.46)	7.21 (7.21)
8d	H	H	$C_{30}H_{21}N_3O_3S$	168–170	0.61	71.25 (71.55)	4.21 (4.20)	8.36 (8.34)
8e	F	Cl	$C_{30}H_{19}ClFN_3O_3S$	233–235	0.72	64.80 (64.81)	3.43 (3.44)	7.56 (7.56)
8f	Cl	Cl	$C_{30}H_{19}Cl_2N_3O_3S$	269–271	0.69	62.96 (62.94)	3.36 (3.35)	7.32 (7.34)

*TLC Solvent system: Chloroform: Methanol: Strong ammonia-(10:5:3).

Table 3
Spectral data of a series of 3-(2-(4-(6,7-disubstituted-benzo[d]thiazol-2-yl)phenylamino)acetyl)−4-hydroxy-1-methyl/phenylquinolin-2(1H)-one (**7a-f** and **8a-f**) derivatives.

Comp	FTIR (KBr disk, Cm^{-1})	^1H NMR (DMSO–d_6, δ, ppm)	Mass: M^+ (*m/z* value)
7a	3456.76 (OH Str), 3385.07 (NH Str), 3078.39 (C–H Str), 1725.94 (*C = O* Str), 1662.45(*C = O* Str), 1639.49 (*C = N* Str)	2.26 (s, 3H, CH_3), 5.64 (s, 2H, CH_2), 6.68–7.78 (m, 11H, Ar-H), 7.67 (s, 1H, NH), 9.85 (s, 1H, OH)	459.24
7b	3498.74 (OH Str), 3356.44 (NH Str), 3089.96 (C–H Str), 1716.03 (*C = O* Str), 1670.10(*C = O* Str), 1631.78 (*C = N* Str)	3.23 (s, 3H, CH_3), 5.59 (s, 2H, CH_2), 6.69–7.88 (m, 11H, Ar-H), 8.59 (s, 1H, NH), 11.26 (s, 1H, OH)	475.96
7c	3469.06 (OH Str), 3350.65 (NH Str), 3078.39 (C–H Str), 1726.29 (*C = O* Str), 1666.27(*C = O* Str), 1637.45 (*C = N* Str)	3.33 (s, 3H, CH_3), 5.60 (s, 2H, CH_2), 6.98–8.03 (m, 11H, Ar-H), 8.62 (s, 1H, NH), 11.04 (s, 1H, OH)	519.03
7d	3468.30 (OH Str), 3382.50 (NH Str), 3084.39 (C–H Str), 1726.28 (*C = O* Str), 1663.55(*C = O* Str), 1635.13 (*C = N* Str)	3.15 (s, 3H, CH_3), 4.99 (s, 2H, CH_2), 7.01–8.08 (m, 12H, Ar-H), 8.57 (s, 1H, NH), 11.30 (s, 1H, OH)	441.14
7e	3476.39 (OH Str), 3338.21 (NH Str), 3083.27 (C–H Str), 1720.62 (*C = O* Str), 1672.90(*C = O* Str), 1638.28 (*C = N* Str)	3.62 (s, 3H, CH_3), 5.09 (s, 2H, CH_2), 6.91–8.08 (m, 10H, Ar-H), 8.50 (s, 1H, NH), 11.45 (s, 1H, OH)	493.11
7f	3478.29 (OH Str), 3372.42 (NH Str), 3071.11 (C–H Str), 1728.13 (*C = O* Str), 1660.22(*C = O* Str), 1639.87 (*C = N* Str)	3.56 (s, 3H, CH_3), 5.06 (s, 2H, CH_2), 6.86–8.00 (m, 10H, Ar-H), 8.53 (s, 1H, NH), 10.64 (s, 1H, OH)	509.07
8a	3426.38 (OH Str), 3384.33 (NH Str), 3079.32 (C–H Str), 1719.37 (*C = O* Str), 1669.32(*C = O* Str), 1635.29 (*C = N* Str)	4.95 (s, 2H, CH_2), 6.66–7.82 (m, 16H, Ar-H), 8.64 (s, 1H, NH), 11.09 (s, 1H, OH)	521.21
8b	3492.74 (OH Str), 3353.21 (NH Str), 3089.96 (C–H Str), 1718.63 (*C = O* Str), 1664.36(*C = O* Str), 1633.17 (*C = N* Str)	5.05 (s, 2H, CH_2), 6.92–8.14 (m, 16H, Ar-H), 8.56 (s, 1H, NH), 10.63 (s, 1H, OH)	537.10
8c	3461.25 (OH Str), 3363.06 (NH Str), 3078.21 (C–H Str), 1727.93 (*C = O* Str), 1665.66(*C = O* Str), 1632.11 (*C = N* Str)	5.57 (s, 2H, CH_2), 6.58–8.12 (m, 16H, Ar-H), 8.74 (s, 1H, NH), 10.86 (s, 1H, OH)	581.52
8d	3468.20 (OH Str), 3382.50 (NH Str), 3084.39 (C–H Str), 1726.31 (*C = O* Str), 1666.42(*C = O* Str), 1636.10 (*C = N* Str)	5.03 (s, 2H, CH_2), 7.12–7.85 (m, 17H, Ar-H), 8.62 (s, 1H, NH), 11.06 (s, 1H, OH)	503.52
8e	3472.76 (OH Str), 3335.23 (NH Str), 3082.20 (C–H Str), 1720.54 (*C = O* Str), 1667.35(*C = O* Str), 1635.44 (*C = N* Str)	4.87 (s, 2H, CH_2), 7.04–8.18 (m, 15H, Ar-H), 8.71 (s, 1H, NH), 10.81 (s, 1H, OH)	556.05
8f	3473.48 (OH Str), 3370.38 (NH Str), 3074.88 (C–H Str), 1723.67 (*C = O* Str), 1661.75(*C = O* Str), 1630.26 (*C = N* Str)	5.16 (s, 2H, CH_2), 6.73–8.09 (m, 15H, Ar-H), 8.54 (s, 1H, NH), 10.39 (s, 1H, OH)	572.32

activity against HT29 cancer cells. However, five compounds were found to be highly active against hepatic (liver) cancer cells, from which compound **7e** (IC_{50} = 7.63, 3.15 and 1.80 µM) presented the most promising anticancer activity. Whereas compounds **7f** (IC_{50} = 5.12, 3.73 and 2.48 µM), **8b** (IC_{50} = 9.75, 6.47 and 3.76 µM), **8e** (IC_{50} = 10.84, 7.57 and 5.00 µM), and **8f** (IC_{50} = 9.37, 4.47 and 2.25 µM) demonstrated moderate to good activity as compared to standard Doxorubicin (IC_{50} = 2.25, 1.48 and 0.827 µM) against WRL68 at 24 h, 48 h, and 72 h respectively.

The present study revealed that among the four human cancer cell lines tested, MCF-7 and WRL68 cells were more sensitive to all the tested compounds. The cytotoxicity screening results evidently indicated that the presence of halogens especially fluoro and/or chloro at 6th and 7th position of benzo[*d*]thiazole nucleus contributed to the increase in anticancer activity, which is in agreement with previously reported studies [37,38]. Anticancer drugs, which are active against MCF-7 and WRL68 cells usually, cause apoptosis through the expression of caspase-3, resulting in generation of reactive oxygen species (ROS) and leading to DNA destruction [39,40]. From literature, it is also known that substitution with electronegative atoms (chloro/fluoro) at 6th and/or 7th position on benzo[*d*]thiazole ring increases the lipophilicity of a molecule, which could be responsible for enhanced cytotoxicity in MTT bioassay [6,7,12,41]. Thus in the ti-

Table 4
Cytotoxic activity data of the newly synthesized compounds (**7a-f** and **8a-f**) against various human cancer cell lines at different time points of drug exposure by MTT assay method.

Compounds	IC_{50} (μM)[a] in EAC[b]			IC_{50} (μM) in MCF-7[b]			IC_{50} (μM) in HT29[b]			IC_{50} (μM) in WRL68[b]		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
7a	21.26	16.02	11.92	13.56	09.76	06.33	56.23	35.26	27.86	25.20	19.25	12.04
7b	43.65	35.88	29.21	25.26	19.03	10.02	39.56	30.22	24.15	29.63	16.53	10.19
7c	35.23	26.56	20.76	31.21	22.88	12.74	61.25	50.03	42.05	22.91	17.38	14.20
7d	19.87	17.99	12.19	25.26	16.04	08.53	71.02	63.05	55.25	23.25	16.09	10.63
7e	11.45	09.20	06.93	05.06	03.33	01.76	26.20	22.36	19.54	07.63	03.15	01.80
7f	09.34	05.40	03.08	10.51	05.78	02.97	25.29	20.59	18.55	05.12	03.73	02.48
8a	18.33	13.88	10.39	23.85	13.67	07.86	58.99	39.65	30.48	28.44	20.34	13.60
8b	31.23	23.86	14.44	40.98	32.09	23.23	47.04	31.28	24.56	09.75	06.47	03.76
8c	29.07	23.67	16.30	34.21	22.74	16.99	57.02	37.76	28.41	25.50	18.14	11.40
8d	28.54	20.84	14.86	33.98	20.66	12.87	40.56	31.87	26.73	33.62	24.00	18.77
8e	22.18	16.90	12.34	11.09	07.08	05.55	38.34	33.56	28.69	10.84	07.57	05.00
8f	06.98	04.44	1.817	02.22	0.954	0.398	10.21	07.20	03.01	09.37	04.47	02.25
Standard[c]	04.10	01.86	0.843	1.60	0.625	0.259	06.14	03.45	01.50	02.25	1.48	0.827

[a] The IC_{50} values of tested compounds at 48 h and 72 h were significantly reduced in comparison with 24 h values.
[b] **EAC** (Ehrlich Ascites Carcinoma cells); **MCF7** (hormone dependant breast cancer cells); **HT29** (human colon carcinoma cells); **WRL68** (hepatic cancer cells).
[c] Standard used was Doxorubicin.

Table 5
In vitro antimicrobial activity data of title compounds (**7a-f** and **8a-f**).

Compound	MIC (μg/ml)					
	Staphylococcus aureus	*Bacillus substilis*	*E. coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Asparagus niger*
7a	64	64	8	16	>128	>128
7b	>128	>128	4	8	64	64
7c	64	>128	4	4	32	64
7d	32	>128	8	4	>128	>128
7e	16	32	0.5	2	32	>128
7f	8	64	0.5	0.5	64	>128
8a	16	16	4	8	32	>128
8b	16	32	2	2	64	>128
8c	32	64	4	8	32	>128
8d	>128	>128	4	2	>128	>128
8e	64	128	0.5	2	32	32
8f	16	16	0.5	1	32	>128
Ampicillin	0.5	0.5	0.5	0.5	ND	ND
Gatifloxacin	0.25	0.25	0.25	0.25	ND	ND
Fluconazole	ND	ND	ND	ND	0.5	0.5

*ND= Not determined.

tle compounds, similar substitutions on the benzo[*d*]thiazole scaffold exhibited enhanced anticancer activity. In addition, it was observed that replacement of *N*-phenyl for *N*-methyl group on 4–hydroxy-quinoline-2-one moiety contributed to increase in anticancer activity. Overall, the anticancer activity profile indicated that the synthesized benzo[*d*]thiazolyl substituted-2-quinolone hybrids exhibited encouraging activity against human breast cell line (MCF-7).

2.3. Antimicrobial activity

All the newly synthesized compounds (**7a-f** and **8a-f**) were screened for their *in-vitro* antimicrobial activity against a panel of microorganisms; Gram positive bacteria *Staphylococcus aureus* (ATCC 11,632), *Bacillus substilis* (ATCC 60,511) and Gram negative bacteria *E. coli* (ATCC 10,536), *Pseudomonas aeruginosa* (ATCC 10,145). For antifungal activity, the title compounds were screened against *Candida albicans* (ATCC 2501) and *Aspergillus niger* (ATCC 1781). Ampicillin, Gatifloxacin and Fluconazole were used as reference drugs for antibacterial and antifungal activities. The MIC values of *in vitro* antimicrobial screening are summarized in Table 5. A systematic analysis of the data revealed that some of the synthesized compounds exhibited enhanced antibacterial activity, especially against Gram negative *E. coli* (ATCC 10,536), *P. aeruginosa* (ATCC 10,145) organisms. All the title compounds showed significant antibacterial activity against *E. coli*. Compounds **7e, 7f, 8e,** and **8f** exhibited excellent antibacterial activity with MIC 0.5 $\mu g.mL^{-1}$against *E. coli*, while moderate to good activity (MIC = 1.0 - 8.0 $\mu g.mL^{-1}$) was observed for the remaining compounds in this series. In the case of *P. aeruginosa*, compound **7f** (MIC = 0.5 $\mu g.mL^{-1}$) displayed significant antibacterial activity, whereas other compounds displayed good to moderate antibacterial activity with MIC values ranging from 1.0 to 16.0 $\mu g.mL^{-1}$. However, moderate activity against *S. aureus* was observed for compound **7f** (MIC = 8.0 $\mu g.mL^{-1}$) while other compounds in this series exhibited little or poor activity against Gram-positive bacteria. With respect to antifungal activities (Table 5), all compounds displayed poor or no activity against *C. albicans* and *A. niger*. The MIC values were ranging from 32.0 to >128.0 $\mu g.mL^{-1}$, while the MIC value of standard drug Fluconazole was 0.5 $\mu g.mL^{-1}$. In general, from the antibacterial activity data, an interesting correlation was observed between the compounds and activity against *E. coli* and *P. aeruginosa*. The activity was considerably enhanced by electron withdrawing substituents like chloro or fluoro at 6th and/or 7th position of benzo[*d*]thiazole ring. However, in the case of compounds **7a-d** and **8a-d**, which have no substitution (R_2 = *H*) at 7th position of benzo[*d*]thiazole ring, slightly decrease in activity was noticed. It was also perceived that replacing *N*-phenyl group in place of *N*-methyl group on 4–hydroxy-quinoline-2-one moiety resulted in equipotent antibacterial activity.

2.4. Molecular docking studies

The epidermal growth factor receptor (EGFR) and transforming growth factor-$\propto$ are one of the earliest characterized members of the growth factor/receptor tyrosine kinase (RTK) family. The EGFR induces cell proliferation and cell differentiation upon binding and activation by one of a number of its known ligands. Members of the EGFR family are frequently overactive in solid tumors [42]. For example, the epidermal growth factor receptor tyrosine kinases (EGFRTK) of the erbB family (which includes erbB1-erbB4) is frequently expressed at high levels in certain carcinomas particularly in breast, colon, and bladder cancers [43]. A number of therapeutic approaches that interfere with aberrant EGFR family signaling are being investigated [44]. A relatively new therapeutic approach to kinase inhibition is the use of ATP-competitive small molecules [45]. From literature, it is well known that anticancer benzo[*d*]thiazole and quinazoline analogs act via competing with ATP for binding at the catalytic domain of tyrosine kinase [7,46].

The *in vitro* cytotoxic activity results especially against MCF-7 were inspiring and in order to validate experimental results further and to explore the possible binding interactions. Molecular docking studies for the title compounds (**7a-f** and **8a-f**) were carried out using Molegro Virtual Docker (MVD-2013, 6.0). Fig. 2 represents the native crystal structure of Erlotinib (AQ4) bound to the active site of EGFRTK obtained from Protein Data Bank (http://www.rscb.org/pdb) with the PDB ID: **1M17** [47]. The EGFR consists of a single polypeptide chain of 1186 amino acids that is expressed on the cell membrane of numerous cell types. An essential feature of the binding site is the conservation of hydrogen bondings, steric and aromatic π-π stacking interactions. The active pocket consisted of amino acid residues such as Val702, Ala719, Lys721, Met742, Thr766, Gln767, Leu768, Met769, Pro770, Gly772, Leu820, Thr830, and Asp831. Hence to identify other residual interactions of the tested compounds, a grid box (include residues within a 10.0 Å radius) large enough to accommodate the active site was constructed. AQ4 being a known inhibitor, the center of this site was considered as the center of search space for docking. The 2D structures of the synthesized ligands were converted to energy minimized 3D structures and were used for *in silico* protein–ligand docking calculations. Docking of the synthesized compounds with the EGFRTK exhibited the well-conserved interactions like hydrogen bonding, hydrophobic bonding, and van der Waals's interactions with one or more amino acid residues of the active pocket. The molecular docking results are summarized in Table 6, from which the following assumptions can be drawn. For the test compounds (**7a-f** and **8a-f**), the MolDock score ranged from –77.7254 to –129.7780 while the Moldock score of standard drug AQ4 was –114.2710. The best poses (orientations) of AQ4 and of the docked compounds are represented in Figs. 2 and 3. It was clearly observed that the nitrogen atom (N-1 of quinazoline) and oxygen atom (methoxyl side chain) of standard drug (AQ4) exhibited distinctive hydrogen bonding with Met769 (2.8387 Å) and Cys773 (3.3126 Å), respectively. Many researchers reported the similar binding mode in protein kinase structures with the high degree of sequence conservation present in the catalytic core of protein kinases are in agreement the present work. Among the tested series, compound **8f** displayed maximum conserved hydrogen bond interactions with the surrounding amino acid residues of the active site. It was observed that the oxygen atom (4–hydroxyl group of 2-quinolone ring) exhibited three hydrogen bonding interactions with hydroxyl (OH) group of Thr830 (2.8306 Å), of Asp831 (3.0922 Å), and amino group (NH) of Asp831 (3.1830 Å). The nitrogen atom on 2-phenyl ring (linker:-Ph–NH–CH_2-) displayed two hydrogen bonding interactions with OH group of Thr830 (3.5802 Å) and Thr766 (2.7066 Å) respectively. Interestingly, The N-3 of the benzo[*d*]thiazole moiety generates much conserved hydrogen bond interactions with the NH spine of Met-769 (3.5167 Å). Normally, this amino acid residue forms very crucial hydrogen bond with N-1 of the adenine ring in ATP and helps to fix it in the binding pocket. This hydrogen bond can be considered as decisive for orienting the benzo[*d*]thiazole ring in the pocket. A second hydrogen bond interactions were observed with carbonyl oxygen atom of 2-quinolone ring and the OH group in side chain of Thr-766 (3.0474 Å), which is located at the beginning of the extended coil stretch deep in the binding cleft. Similarly compound **7f** also displayed five hydrogen bonding interactions as depicted in Fig. 3. In the case of compounds **7e** and **8e**, prominent hydrogen bonding interactions were observed, that is between nitrogen atom (*N*-1) of 2-quinolone ring and OH group of the residue Thr 766 (3.0558 Å and 2.7788 Å), nitrogen atom of the linker (-Ph–NH–CH_2-) and NH group of Lys 721 (3.4616 Å and 3.1567 Å) and carbonyl oxygen atom of 2-quinolone ring with the OH group of Thr 830 (2.7415 Å and 2.8145 Å), respectively. Nevertheless, the less effective compounds **8b** (3.2038 Å), **8c** (3.1357 Å), and **8d** (3.1764 Å), displayed a prominent hydrogen bonding interactions with NH group of Cys773 residue with the oxygen atom of a keto group on the 3rd position of 2-quinolone nucleus. Besides hydrogen bonding interactions, the docking results of the active compounds also exhibited steric and aromatic π-π stacking interactions with the various amino acid residues in the active site of **1M17**, which are essential for the inhibition of EGFRTK [47]. The above results demonstrate that these hybrid compounds could be effective inhibitors of EGFRTK. Thus, the *in-silico* results provide an insight into the binding mode of benzo[*d*]thiazolyl substituted-2-quinolone hybrids in the active site of **1M17**.

2.5. Conclusion

In this paper, the synthesis, spectral studies, and biological evaluation of a series of 3-(2-(4-(substituted-benzo[*d*]thiazol-2-yl)phenylamino)acetyl)–4–hydroxy-1-methyl/phenyl quinolin-2(1*H*)-one (**7a-f** and **8a-f**) derivatives are reported. The *in vitro* anticancer results obtained indicated that the compound **8f** was most active hybrid in the series and from the SAR studies it was concluded that the presence of electronegative groups (Cl/F) on the benzo[*d*]thiazole nucleus was favorable and the presence of quinazoline moiety is also equally responsible for substantial increase in anticancer activity. Molecular binding interaction pattern of the title compounds with the target enzyme (EGFR tyrosine kinsae) was effectually generated with the help of docking simulations and the results indicate that the compounds **8f** and **7f** displayed most relevant conserved hydrogen bond interactions requisite for **1M17** inhibition. Moreover, in many respects the SAR profile exhibited by these compounds is almost similar to that shown by the previously reported quinazoline series of EGFRTK inhibitors. Hence, the molecular hybridization strategy chosen in the current study to combine key pharmacophoric features (2-phenyl benzo[*d*]thiazole and substituted 2-quinolone) connected with suitable linker was fruitful. The *in vitro* antimicrobial screening of the title compounds **7e, 7f, 8e,** and **8f** exhibited notable antibacterial activity especially against Gram-negative bacterium (*E. coli*). In conclusion, the novel series of benzo[*d*]thiazolyl substituted-2-quinolone hybrids resulted in identifying a hit molecule **8f**, which could be explored further to develop potential anticancer and antibacterial agents.

3. Experimental

3.1. Synthetic protocol

All research chemicals were purchased from Sigma–Aldrich (St. Louis, Missouri, USA) or Lancaster Co. (Ward Hill, MA, USA) and used as such for the reactions. Solvents except laboratory reagent

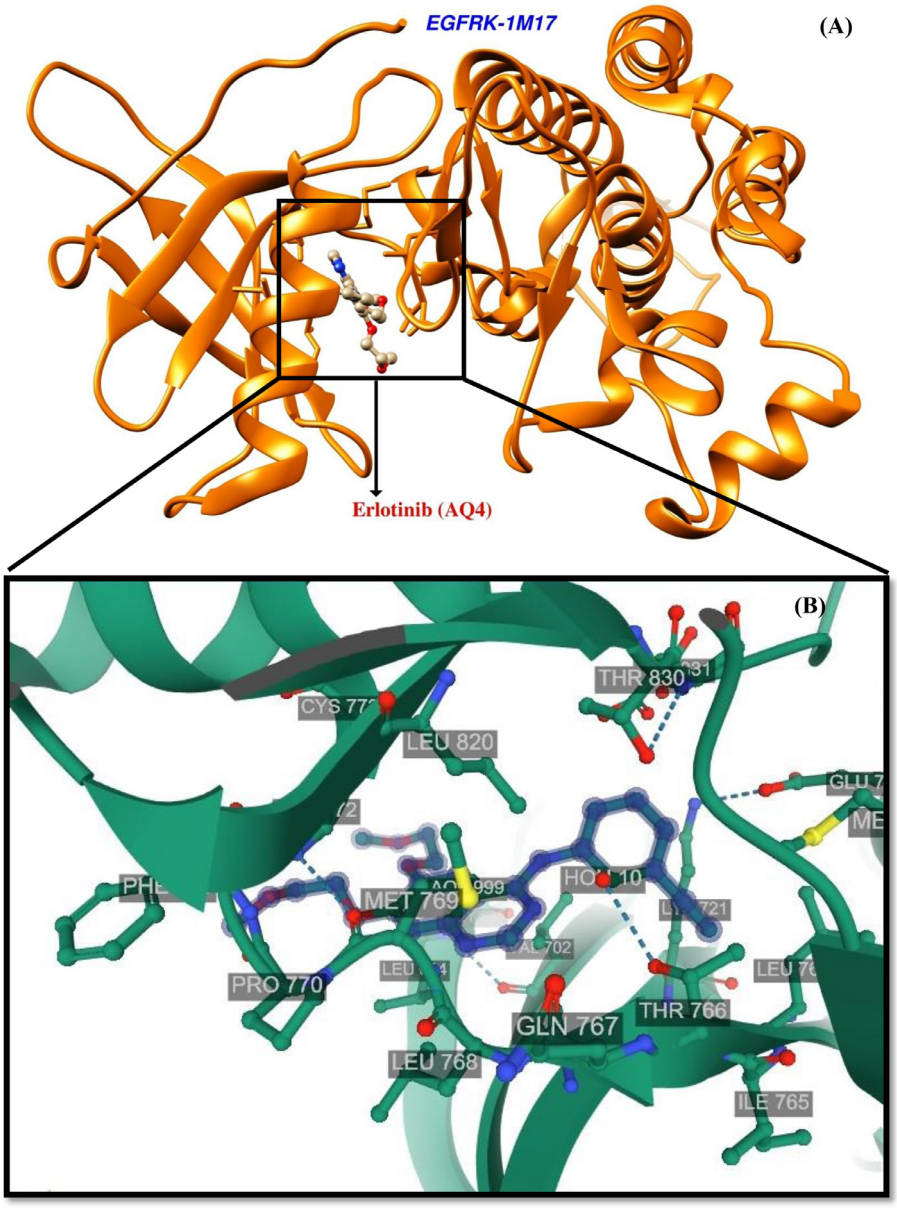


Fig. 2. (**A**) Secondary structure (complete protein) epidermal growth factor receptor tyrosine kinase (EGFRTK, PDBID: 1M17) in complex with inhibitor (AQ4: Erlotinib); (**B**) showing the binding orientation of AQ4 forming 1H bond with Met769 and 1H bond with Cys773.

grade were dried and purified according to the literature when necessary. The reference drugs used in this work was received as a gift sample from Fourrtis (India) Lab. Pvt. Ltd., Chennai (Tamilnadu, India). The progress of reactions and purity of compounds were monitored by thin-layer chromatography (TLC) on pre-coated aluminum sheets with GF_{254} silica gel plates procured from E. Merck and Co. (Darmstadt, Germany). Methanol (12%) in dichloromethane was used as mobile phase and short wavelength UV radiations as well as iodine vapours were used as visualizing agent.

Melting points of synthesized compounds were determined in Veego (VMP-MP) melting point apparatus and were uncorrected. IR spectra were recorded on ThermoNicolet IR200 FT-IR Spectrometer (Madison, WI, USA) by using KBr pellets and Brucker Alpha I FTIR Spectrophotometer. The ^{1}H NMR spectra were recorded on Bruker Avance III 400 NMR spectrometer (Bruker, Rheinstetten/Karlsruhe, Germany) using appropriate solvent at Sophisticated Analytical and Instrumentation Facility (SAIF), Punjab University (Chandigarh, India). Chemical shifts are reported in δ ppm units with respect to TMS as internal standard. Mass spectra were recorded on Shimadzu LC MS-2010A at Quest Research and Training Institute Private Limited, (Bangalore, India).

3.2. General procedure for synthesis of N-(substituted phenyl)−4-nitrobenzamide (1a-f)

A mixture of substituted aniline (0.02 mol) and 4-nitrobenzoyl chloride (0.02 mol, 0.371 g) and 50 mL of pyridine (The excess of solvent is used to get pure compound) was refluxed for 2–5 h. The reaction mixture was cooled to room temperature and poured into ice-cold water. The precipitate formed was collected, washed with ice-cold water, and recrystallized from either ethyl alcohol or methyl alcohol.

N-(4-fluorophenyl)−4-nitrobenzamide (**1a**): It is light green color crystalline needles (EtOH); yield 4.3 g (82.69%); mp 176–178 °C;

Table 6
Molecular docking results based on Moldock score, E-inter, hydrogen bonding interaction, hydrophobic/steric interaction, Docking scores and Rerank scores of the title compounds by MVD-2013 (6.0) against 1M17.

Compds	Moldock Score (Kcal/mol)	E-Inter (protein–ligand) (Kcal/mol)	Residues involved in hydrophobic and steric interactions (within 5 Å)	Residues involved in hydrogen bonding	H-Bonds No.	Heavy atoms count (Kcal/mol)	LE1	LE3	Docking Score (Kcal/mol)	Rerank score (Kcal/mol)	
7a	−88.7340	−111.281	Leu694, Ala719, Glu738, Thr766, Leu768, Met769, Gly772, Leu820	––	00	0.0	33	−2.6889	−0.8373	−511.185	−27.6334
7b	−101.5240	−125.862	Val702, Lys721, Met742, Thr766, Gln767, Met769, Leu820	Cys773	01	−0.5681	33	−3.0764	−1.0360	−535.941	−34.1897
7c	−77.7254	−105.273	Leu694, Ala719, Met769	Gly772	01	−0.6800	33	−2.3553	−0.8252	−511.824	−27.2316
7d	−89.4629	−114.103	Leu694, Leu768, Met769, Gly772, Leu820	Thr766	01	−1.5403	32	−2.7957	−0.5223	−532.235	−16.7163
7e	−117.8740	−146.335	Ala719, Cys751, Gln767, Met769, Leu820	Lys721, Thr766, Thr830	03	−5.6917	34	−3.4668	−1.2522	−584.646	−42.5758
7f	−120.6290	−150.548	Val702, Met742, Thr766, Gln767, Leu820	Thr766, Thr830, Asp831	05	−9.2427	39	−3.0930	−2.2980	−584.646	−89.6235
8a	−101.0260	−114.103	Val702, Ala719, Met742, Leu768, Met769, Leu820	–––	00	0.0	38	−2.6585	−0.9297	−519.330	−35.3300
8b	−105.1150	−131.158	Val702, Ala719, Met742, Gln767, Leu768, Met769, Leu820	Cys773	01	−1.9806	38	−2.7661	−0.8585	−526.940	−32.6260
8c	−108.3960	−132.463	Val702, Gln767, Leu768, Met769, Leu820	Cys773	01	−2.3213	38	−2.8525	−1.6943	−531.134	−64.3870
8d	−113.6400	−143.527	Val702, Ala719, Leu768, Met769, Leu820	Cys773	01	−2.1175	37	−3.0713	−2.2006	−563.963	−81.4240
8e	−117.8990	−148.116	Ala719, Cys751, Thr766, Gln767, Met769, Leu820	Lys721, Thr766, Thr830	03	−7.2163	39	−3.0230	−2.1324	−569.095	−83.1648
8f*	−129.7780	−161.837	Val702, Ala719, Met742, Thr766, Gln767, Leu768, Met769, Leu820, Asp831	Thr766, Met769, Thr830, Asp831	07	−9.5463	34	−3.8170	−2.7854	−592.379	−94.7051
AQ4	−114.2710	−131.574	Pro770, Gly772	Met769, Cys773	02	−3.93697	29	−3.9403	−3.2729	−579.7700	−94.9144

*Compound exhibited high binding energy (Moldock score); H. Bonds: Hydrogen bonding energy between protein and ligand; LE1(Ligand Efficiency): MolDock score divided by heavy atoms count; LE3: Rerank score divided by heavy atoms count; AQ4: Erlotinib (4-Anilinoquinazoline derivative).

IR (KBr, ν, cm^{-1}): 3325.45 (N–H str) 3078.24 (-CH Str), 1627.30 (-*C* = *O* str of amide); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 8.29 (s, 1H, NH), 8.03–6.92 (m, 8H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 172.54 (*C* = *O* carbon of amide), 160.41, 155.39, 137.00, 135.10, 128.32, 125.96, 123.40, 116.43; ESI MS: *m/z* 260.31; calcd. 260.22.

N-(4-chlorophenyl)–4-nitrobenzamide(**1b**): It is white crystalline needles (EtOH); yield 2.5 g (93.63%); mp 232–234 °C; IR (KBr, ν, cm^{-1}): 3345.39 (N–H str) 3014.02 (-CH Str), 1645.13 (-*C* = *O* str of amide); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 8.12 (s, 1H, NH), 7.92–7.08 (m, 8H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 171.15 (*C* = *O* carbon of amide), 163.32, 155.33, 138.35, 135.14, 127.02, 125.34, 122.41, 116.44; ESI MS: *m/z* 276.11; calcd. 276.03.

N-(4-bromophenyl)–4-nitrobenzamide(**1c**): It is light brown crystalline powder (MeOH); yield 2.7 g (84.37%); mp 217–219 °C; IR (KBr, ν, cm^{-1}): 3278.30 (N–H str) 3021.43 (-CH Str), 1643.57 (-*C* = *O* str of amide); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 8.76 (s, 1H, NH), 8.02–6.75 (m, 8H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 171.45 (*C* = *O* carbon of amide), 162.84, 155.30, 137.30, 135.34, 127.65, 125.22, 122.11, 116.91; ESI MS: *m/z* 320.05; calcd. 319.98.

4-nitro-N-phenylbenzamide(**1d**): It is white crystalline powder (EtOH); yield 2.0 g (82.64%); mp 181–183 °C; IR (KBr, ν, cm^{-1}): 3223.56 (N–H str) 2968.43 (-CH Str), 1639.97 (-*C* = *O* str of amide); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 8.14 (s, 1H, NH), 8.12–6.58 (m, 9H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 172.60 (*C* = *O* carbon of amide), 162.30, 155.32, 137.32, 125.22, 122.65, 116.51; ESI MS: *m/z* 242.05; calcd. 242.07.

N-(3–chloro–4-fluorophenyl)–4-nitrobenzamide(**1e**): It is light green crystalline powder (EtOH); yield 1.5 g (54.42%); mp 202–204 °C; IR (KBr, ν, cm^{-1}): 3331.45 (N–H str) 3123.34 (-CH Str), 1660.05 (-*C* = *O* str of amide); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 8.23 (s, 1H, NH), 7.92–7.01 (m, 7H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 169.58 (*C* = *O* carbon of amide), 161.54, 153.69, 136.11, 127.72, 125.20, 122.37, 119.65, 117.45; ESI MS: *m/z* 294.02; calcd. 294.04.

N-(3,4-dichlorophenyl)–4-nitrobenzamide(**1f**): It is white crystalline compound (EtOH); yield 2.1 g (67.74%); mp 197–199 °C; IR (KBr, ν, cm^{-1}): 3261.76 (N–H str) 3087.34 (-CH Str), 1651.03 (-*C* = *O* str of amide); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 8.43 (s, 1H, NH), 7.95–6.96 (m, 7H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 168.43 (*C* = *O* carbon of amide), 161.39, 153.69, 135.94, 126.99, 125.22, 123.18, 119.61, 117.65; ESI MS: *m/z* 310.01; calcd. 309.99.

3.3. General procedure for synthesis of N-(substituted phenyl)–4-nitrobenzothioamide (2a-f)

A mixture of one of the N-(substituted phenyl)–4-nitrobenzamide (**1a**-**f**, 0.01 mol) and Lawesson's reagent (0.6 mol Equivalent (Eq)) in 30 mL of hydroxymethylphospharamide (HMPA) was stirred at 100 °C for 6–9 h. The reaction mass was

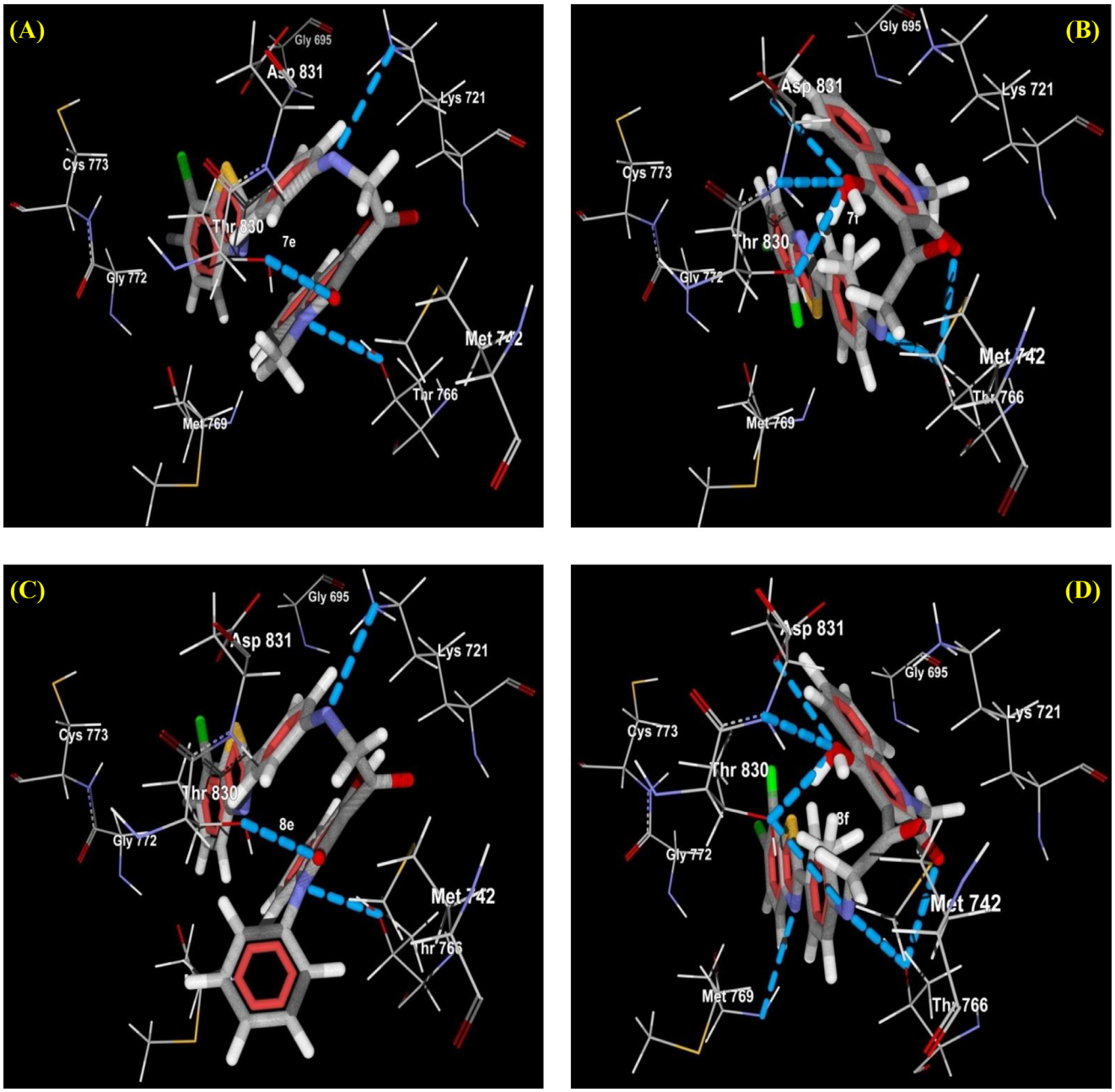


Fig. 3. Showing the compounds docked in best of its conformation into the binding site of EGFRTK (1M17); broken lines (blue color) indicate hydrogen bonding interactions. (**A**) Binding mode of compound **7e** forming 1H bond with Lys721, 1H bond with Thr766 and 1H bond with Thr830; (**B**) Binding mode of compound **7f** forming 2H bonds with Thr766, 1H bond with Thr830 and 2H bonds with Asp831; (**C**) Binding mode of compound **8e** forming 1H bond with Lys721, 1H bond with Thr766 and 1H bond with Thr830; (**D**) Binding mode of compound **8f** forming 2H bonds with Thr766, 1H bond with Met769, 2H bonds with Thr830 and 2H bonds with Asp831. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

then poured into ice-cold water. Thus obtained desired products **2a-f** were collected as crystalline solids and washed with ice-cold water, dried, and recrystallized from methanol.

*3.4. General procedure for jacobson's synthesis of 6,7-substituted-2-(4-nitrophenyl) benzo[*d*]thiazole (3a-f) [34]*

N-(substituted phenyl)−4-nitrobenzothioamide (**2a-f**, 0.05 mol) were drenched in 5 mL of ethanol, to which 30% sodium hydroxide (NaOH) solution (8 mol Eq) was slowly added with constant stirring. The mixture was diluted with distilled water to provide a final concentration of 10% aqueous NaOH. Aliquots of this mixture (5 mL) were carefully added at 1 min intervals to a well stirred solution of potassium ferricynide (4 mol Eq) in hot water maintained at 90 °C. The reaction mixture was further heated for 1–2 h and then cooled to the room temperature. The products (**3a-f**) formed were collected, washed with ice-cold water, and recrystallized from methanol.

6-fluoro-2-(4-nitrophenyl) benzo[d]thiazole (**3a**): Yield 1.19 g (69.20%); mp 188–190 °C; IR (KBr, ν, cm^{-1}): 3072.58 (-CH Str), 1662.18 (-*C* = *N* str); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 7.95–6.96 (m, 7H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 168.56,

153.50, 148.64, 138.41, 128.19, 126.22, 123.39, 122.25; ESI MS: *m/z* 273.96; calcd. 274.02.

6–chloro–2-(4-nitrophenyl) benzo[d]thiazole (**3b**): Yield 2.10 g (72.66%); mp 213–215 °C; IR (KBr, ν, cm^{-1}): 3094.21 (-CH Str), 1658.68 (-*C* = *N* str); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 8.04–6.94 (m, 7H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 166.45, 154.34, 148.33, 136.35, 128.12, 125.20, 123.34, 121.21; ESI MS: *m/z* 290.06; calcd. 289.99.

6–bromo–2-(4-nitrophenyl) benzo[d]thiazole (**3c**): Yield 2.60 g (77.84%); mp 234–235 °C; IR (KBr, ν, cm^{-1}): 3156.54 (-CH Str), 1652.61 (-*C* = *N* str); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 7.84–7.04 (m, 7H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 167.35, 154.31, 148.31, 136.32, 128.52, 124.54, 119.01; ESI MS: *m/z* 334.02; calcd. 333.94.

2-(4-nitrophenyl)benzo[d]thiazole (**3d**): Yield 1.80 g (70.31%); mp 226–228 °C (lit[35] 229–231 °C); IR (KBr, ν, cm^{-1}): 3124.68 (-CH Str), 1656.39 (-*C* = *N* str); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 8.39–7.47 (m, 8H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 148.64, 153.50, 164.56, 138.41, 135.04, 128.19, 126.80, 126.11, 124.30, 123.39, 122.25; ESI MS: *m/z* 256.06; calcd. 256.03.

7–chloro–6-fluoro-2-(4-nitrophenyl)benzo[d]thiazole (**3e**): Yield 2.45 g (79.36%); mp 140–142 °C; IR (KBr, ν, cm^{-1}): 3074.43 (-CH Str), 1639.34 (-*C* = *N* str); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 8.12–7.01 (m, 6H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 168.32, 152.54, 149.43, 145.78, 139.22, 135.62, 128.20, 122.50, 114.32; ESI MS: *m/z* 308.03; calcd. 307.98.

6,7-dichloro-2-(4-nitrophenyl)benzo[d]thiazole (**3f**): Yield 2.25 g (69.02%); mp 164–166 °C; IR (KBr, ν, cm^{-1}): 3088.41 (-CH Str), 1641.03 (-*C* = *N* str); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 8.11–6.86 (m, 6H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 167.47, 152.78, 148.10, 144.54, 140.21, 134.27, 128.65, 124.17, 122.30; ESI MS: *m/z* 323.92; calcd. 323.95.

3.5. General procedure for synthesis of 6,7-substituted-2-(4-aminophenyl)benzo[d]*thiazole (4a-f) [48]*

A mixture of an appropriately substituted-2-(4-nitrophenyl) benzo[*d*]thiazole (one of **3a**-**f**, 0.015 mol) and tin (II) chloride dihydrate (0.075 mol) in 50 mL boiling ethanol was stirred under nitrogen environment for 3–5 h. The excess ethanol was removed by vacuum evaporation using rota-evaporator and the residue formed was successively extracted with 75 ml of ethyl acetate (3 times). The combined organic fraction was further thoroughly washed first with 50 mL of 2 M aqueous NaOH (3 times) followed by 50 mL of distilled water (2 times). To the resultant organic fraction, anhydrous Na_2SO_4 was added, filtered, and the filtrate was evaporated *in-vacuum* to yield the desired compounds **4a-f**.

4-(6-fluorobenzo[d]thiazol-2-yl)benzenamine (**4a**): Yield 1.63 g (66.80%); R_f 0.25; mp 171–173 °C; IR (KBr, ν, cm^{-1}): 3425.63, 3420.24 (-NH_2 Str), 3125.35 (-CH Str), 1654.11 (-*C* = *N* str); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 7.97 (m, 1H, Ar-H), 7.87 (s, 2H, NH_2), 7.38–6.67 (m, 6H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 168.42, 159.39, 153.64, 151.42, 133.46, 128.20, 125.36, 124.04, 121.49, 120.35, 113.27; LCMS: *m/z* 243.99; Calcd. 244.05; Anal. Calcd: C, 63.92; H, 3.71; N, 11.47. Found: C, 63.84; H, 3.48; N, 11.37.

4-(6-chlorobenzo[d]thiazol-2-yl)benzenamine (**4b**): Yield 0.96 g (36.92%); R_f 0.64; mp 194–196 °C; IR (KBr, ν, cm^{-1}): 3498.74, 3489.29 (-NH_2 Str), 3077.67 (-CH Str), 1674.80 (-*C* = *N*); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 7.91 (m, 1H, Ar-H), 7.40 (s, 2H, NH_2), 7.29–7.20 (m, 1H, Ar-H), 7.04–6.81 (m, 5H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 168.11, 158.23, 153.61, 152.49, 133.44, 128.60, 125.36, 124.34, 122.51, 119.88, 114.71; LCMS: *m/z* 259.86; Calcd. 260.02; Anal. Calcd: C, 59.88; H, 3.48; N, 10.74. Found: C, 59.85; H, 3.29; N, 10.65.

4-(6-bromobenzo[d]thiazol-2-yl)benzenamine (**4c**): Yield 1.07 g (35.31%); R_f 0.34; mp 161–163 °C; IR (KBr, ν, cm^{-1}): 3469.06, 3450.57 (-NH_2 Str), 3078.69 (-CH Str), 1662.20 (-*C* = *N*); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 7.65–7.60 (m, 1H, Ar-H), 7.35 (s, 2H, NH_2), 7.30–6.71 (m, 6H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 170.11, 156.21, 154.29, 150.18, 133.42, 128.22, 125.36, 124.65, 121.77, 121.05, 113.28; LCMS: *m/z* 303.93; Calcd. 303.97; Anal. Calcd: C, 51.16; H, 2.97; N, 9.18. Found: C, 51.04; H, 3.01; N, 9.10.

2-(4-aminophenyl)benzo[d]thiazole (**4d**): Yield 1.78 g (78.76%); R_f 0.79; mp 157–159 °C; IR (KBr, ν, cm^{-1}): 3454.17, 3433.57 (-NH_2 Str), 3080.10, (-CH Str), 1659.11 (-*C* = *N*); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 8.08–7.01 (m, 8H, Ar-H), 7.57 (s, 2H, NH_2); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 168.12, 153.86, 151.91, 151.85, 133.72, 128.62, 125.92, 124.07, 121.64, 121.52, 120.31, 113.58, 113.54; LCMS: *m/z* 225.98; Calcd. 226.04; Anal. Calcd: C, 69.00; H, 4.45; N, 12.38. Found: C, 68.70; H, 4.39; N, 12.36.

4-(7–chloro–6-fluorobenzo[d]thiazol-2-yl)benzenamine (**4e**): Yield 1.60 g (57.55%); R_f 0.59; mp 180–182 °C; IR (KBr, ν, cm^{-1}): 3433.45, 3426.20 (-NH Str), 3083.46, (-CH Str), 1648.10 (-*C* = *N*); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 7.91 (s, 2H, NH_2), 7.22–7.18 (m, 1H, Ar-H), 7.16–6.93 (m, 5H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 167.93, 153.39, 152.01, 133.71, 128.88, 125.96, 124.00, 121.51, 120.38, 114.67; LCMS: *m/z* 277.97; Calcd. 278.01; Anal. Calcd: C, 56.02; H, 2.89; N, 10.05. Found: C, 56.00; H, 2.37; N, 10.04.

4-(7–chloro–6-fluorobenzo[d]thiazol-2-yl)benzenamine (**4f**): Yield 1.45 g (49.32%); R_f 0.91; mp 189–191 °C; IR (KBr, ν, cm^{-1}): 3477.73, 3462.41 (-NH_2 Str), 3101.12, (-CH Str), 1653.47 (-*C* = *N*); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 8.02 (m, 1H, Ar-H), 7.85 (s, 2H, NH_2), 7.77–7.04 (m, 5H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 168.01, 154.30, 151.67, 133.35, 128.09, 125.50, 124.08, 121.11, 120.84, 114.39; LCMS: *m/z* 294.04; Calcd. 293.98; Anal. Calcd: C, 52.89; H, 2.73; N, 9.49. Found: C, 52.65; H, 2.56; N, 9.42.

3.6. General procedure for synthesis of 3-(2-bromoacetyl)–4–hydroxy-1-methyl/phenyl quinolin-2(1H)-one (5 and 6) [35]

As shown in Scheme 2, these compounds were prepared by dissolving 3-acetyl-4–hydroxy-1-methyl/phenyl quinolin-2(1H)-one (0.065 mol) in glacial acetic acid (50 mL) and gently heated at 80 °C with constant stirring for 30 min. The equimolar quantity of bromine (0.065 mol) in glacial acetic acid (10 mL) was slowly added in a drop wise manner for a period of 1 h and heating of the solution was continued until a slight change in the color of the solution was observed. The reaction mixture was cooled to room temperature. The products (**5** and **6**) were obtained as yellow crystalline solid and recrystallized from ethanol.

3-(2-bromoacetyl)–4–hydroxy-1-methylquinolin-2(1H)-one (**5**): Yield 12.4 g (64%), mp 172–174 °C, IR (KBr, ν, cm^{-1}): 3469.02 (-OH), 2985.31(-CH str), 1737.08 (-*C* = *O* str of $COCH_2Br$), 1658.22 (-*C* = *O* str of amide); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 15.68 (s, 1H, OH), 8.68–7.22 (m, 4H, Ar-H), 3.23 (s, 2H, CH_2), 2.64 (s, 3H, *N*–CH_3); ^{13}C NMR (101 MHz, DMSO–d_6, δ, ppm): 201.03 (*C* = *O* carbon of **C**OCH_2Br), 167.07 (*C* = *O* of 2-quinolone), 137.06, 128.87, 126.36, 124.45, 118.17, 115.60, 107.04 (Aromatic carbons), 33.45 (*N*–CH_3), 35.98 (CH_2Br); LCMS: *m/z* 294.92; calcd. 294.98.

3-(2-bromoacetyl)–4–hydroxy-1-phenylquinolin-2(1H)-one (**6**): Yield 11.6 g (50%), mp 264–266 °C, IR (KBr, ν, cm^{-1}): 3478.43 (-OH Str), 2990.30 (-CH str), 1732.05 (-*C* = *O* str of $COCH_2Br$), 1655.33 (-*C* = *O* str of amide); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 16.89 (s, 1H, OH), 8.28–6.12 (m, 9H, Ar-H), 2.82 (s, 2H, CH_2); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 205.09 (*C* = *O* carbon of **C**OCH_2Br), 174.39 (*C* = *O* of 2-quinolone), 163.42, 141.09, 137.00,

135.11, 129.77, 129.07, 128.24, 125.66, 120.47, 116.93, 114.42, 104.47, 36.03 (CH_2Br); LCMS: *m/z* 256.73; calcd. 257.00.

3.7. General procedure for synthesis of 3-(2-(4-(6,7-substituted-benzo[d]thiazol-2-yl)phenyl aminoacetyl)−4−hydroxy-1-methyl/phenyl quinolin-2(1H)-one (7a-f and 8a-f)

As illustrated in **Scheme 2**, a mixture of an appropriately substituted-2-(4-amino phenyl)benzo[*d*]thiazole (one of **4a**-**f**, 0.004 mol) and 3-(2-bromoacetyl)−4−hydroxy-1-methyl/phenylquinolin-2(1H)-one (**5**/**6**, 0.004 mol) was dissolved in 45 mL of glacial acetic acid with constant stirring. The reaction mixture was then heated at reflux temperature for about 6–13 h. The progress of the reaction was monitored by the TLC. The reaction mixture was allowed to attain the room temperature. The solvent was removed under vacuum. The residue was dissolved in ethyl acetate, washed twice with water, and dried (Na_2SO_4). Further, the title compounds were purified by removal of the solvent followed by column chromatography using 200–400 mesh silica gel eluting with methanol (12%) in dichloromethane.

3-(2-(4-(6-fluorobenzo[d]thiazol-2-yl)phenylamino)acetyl)−4−hydroxy-1-methylquinolin-2(1H)-one (**7a**): Yield 3.25 g (70.80%); R_f 0.57; mp 194–196 °C; IR (KBr, ν, cm^{-1}): 3456.76 (-OH Str), 3385.07 (-NH Str), 3078.39 (-CH Str), 1725.94 (*C* = *O* Str), 1662.45 (*C* = *O* Str), 1639.49 (*C* = *N* Str); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 9.85 (s, 1H, OH), 7.67 (s, 1H, NH), 6.68–7.78 (m, 11H,Ar-H), 5.64 (s, 2H, CH_2), 0.26 (s, 3H, CH_3); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 193.48, 167.27, 165.37, 158.51, 153.33, 151.98, 151.22, 134.67, 128.66, 125.44, 124.75, 123.60, 122.30, 119.32, 113.45, 113.01, 109.28, 56.64, 27.30; LCMS: *m/z* 459.06; Calcd. 459.11; Anal. Calcd: C, 65.35; H, 3.95; N, 9.14. Found: C, 65.35; H, 3.92; N, 9.16.

3-(2-(4-(6-chlorobenzo[d]thiazol-2-yl)phenylamino)acetyl)−4−hydroxy-1-methylquinolin-2(1H)-one (**7b**): Yield 3.50 g (73.68%); R_f 0.53; mp 176–178 °C; IR (KBr, ν, cm^{-1}): 3498.74 (OH Str), 3356.44 (NH Str), 3089.96 (-CH Str), 1716.03 (*C* = *O* Str), 1670.10(*C* = *O* Str), 1631.78 (*C* = *N* Str); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 11.26 (s, 1H, OH), 8.59 (s, 1H, NH), 7.88–6.69 (m, 11H,Ar-H), 5.59 (s, 2H, CH_2), 3.23 (s, 3H, CH_3); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 192.05, 168.06, 166.22, 158.18, 154.20, 151.87, 151.04, 133.61, 128.26, 125.05, 124.88, 123.23, 122.11, 119.35, 114.27, 114.03, 110.23, 57.70, 28.28; LCMS: *m/z* 475.00; Calcd. 475.08; Anal. Calcd: C, 63.09; H, 3.81; N, 8.83. Found: C, 63.03; H, 3.79; N, 8.89.

3-(2-(4-(6-bromobenzo[d]thiazol-2-yl)phenylamino)acetyl)−4−hydroxy-1-methylquinolin-2(1H)-one (**7c**): Yield 3.12 g (60.81%); R_f 0.47; mp 209–211 °C; IR (KBr, ν, cm^{-1}): 3469.06 (-OH Str), 3350.65 (NH Str), 3078.39 (-CH Str), 1726.29 (*C* = *O* Str), 1666.27(*C* = *O* Str), 1637.45 (*C* = *N* Str); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 11.04 (s, 1H, OH), 8.62 (s, 1H, NH), 8.03–6.98 (m, 11H,Ar-H), 5.60 (s, 2H, CH_2), 3.33 (s, 3H, CH_3); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 192.67, 168.52, 163.19, 157.82, 153.42, 151.73, 151.38, 133.49, 128.41, 125.41, 124.05, 123.62, 122.30, 119.38, 113.62, 113.21, 110.39, 57.04, 26.37; LCMS: *m/z* 518.97; Calcd. 519.03; Anal. Calcd: C, 57.70; H, 3.49; N, 8.07. Found: C, 57.68; H, 3.43; N, 8.10.

3-(2-(4-(benzo[d]thiazol-2-yl)phenylamino)acetyl)−4−hydroxy-1-methylquinolin-2(1H)-one (**7d**): Yield 3.60 g (81.63%); R_f 0.57; mp 154–156 °C; IR (KBr, ν, cm^{-1}): 3468.30 (OH Str), 3382.50 (NH Str), 3084.39 (-CH Str), 1726.28 (*C* = *O* Str), 1663.55(*C* = *O* Str), 1635.13 (*C* = *N* Str); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 10.30 (s, 1H, OH), 8.57 (s, 1H, NH), 8.08–7.01 (m, 12H, Ar-H), 4.99 (s, 2H, CH_2), 3.15 (s, 3H, CH_3); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 194.62, 168.62, 163.39, 158.42, 153.00, 151.53, 134.53, 128.62, 125.86 124.74, 123.62, 122.53, 119.44, 113.34, 109.29 56.31, 27.86; LCMS: *m/z* 441.10; Calcd. 441.11; Anal. Calcd: C, 68.01; H, 4.34; N, 9.52. Found: C, 67.99; H, 4.34; N, 9.51.

3-(2-(4-(7−chloro−6-fluorobenzo[d]thiazol-2-yl)phenylamino)acetyl)−4−hydroxy-1-methylquinolin-2(1H)-one (**7e**): Yield 3.90 g (79.10%); R_f 0.41; mp 203–205 °C; IR (KBr, ν, cm^{-1}): 3478.06 (OH Str), 3278.06 (NH Str), 3098.39 (-CH Str), 1723.23 (*C* = *O* Str), 1678.78 (*C* = *O* Str), 16.38.57 (*C* = *N* Str); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 11.45 (s, 1H, OH), 8.50 (s, 1H, NH), 8.08–6.91 (m, 10H, Ar-H), 5.09 (s, 2H, CH_2), 3.62 (s, 3H, CH_3); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 193.33, 167.22, 165.30, 159.45, 153.31, 151.67, 134.64, 128.62, 125.41, 124.72, 123.61, 122.33, 119.45, 113.56, 115.76, 109.24, 57.62, 28.31; LCMS: *m/z* 492.95; Calcd. 493.07; Anal. Calcd: C, 60.79; H, 3.47; N, 8.51. Found: C, 60.78; H, 3.44; N, 8.49.

3-(2-(4-(6,7-dichlorobenzo[d]thiazol-2-yl)phenylamino)acetyl)−4−hydroxy-1-methylquinolin-2(1H)-one (**7f**): Yield 3.33 g (65.42%); R_f 0.77; mp 245–247 °C; IR (KBr, ν, cm^{-1}): 3478.29 (OH Str), 3372.42 (NH Str), 3071.11 (-CH Str), 1728.13 (*C* = *O* Str), 1660.22 (*C* = *O* Str), 1639.87 (*C* = *N* Str); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 10.64 (s, 1H, OH), 8.53 (s, 1H, NH), 8.00–6.86 (m, 10H, Ar-H), 5.06 (s, 2H, CH_2), 3.56 (s, 3H, CH_3); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 193.37, 167.18, 164.12, 159.30, 154.56, 151.90, 151.27, 134.34, 128.65, 125.76, 124.65, 123.33, 122.98, 119.19, 113.83, 113.33, 110.20, 56.24, 28.96; LCMS: *m/z* 508.93; Calcd. 509.04; Anal. Calcd: C, 58.83; H, 3.36; N, 8.23. Found: C, 58.85; H, 3.32; N, 8.20.

3-(2-(4-(6-fluorobenzo[d]thiazol-2-yl)phenylamino)acetyl)−4−hydroxy-1-phenylquinolin-2(1H)-one (**8a**): Yield 2.90 g (55.02%); R_f 0.39; mp 222–224 °C; IR (KBr, ν, cm^{-1}): 3444.45 (OH Str), 3346.34 (NH Str), 3139.13 (-CH Str), 1717.14 (*C* = *O* Str), 1675.77 (*C* = *O* Str), 1637.17 (*C* = *N* Str); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 11.09 (s, 1H, OH), 8.64 (s, 1H, NH), 7.82–6.66 (m, 16H, Ar-H), 4.95 (s, 2H, CH_2); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 192.55, 168.53, 167.06, 151.45, 149.41, 138.51, 136.28, 136.01, 128.97, 128.60, 127.37, 125.29, 124.70, 122.76, 122.16, 119.32, 113.41, 113.16, 109.38, 57.39; LCMS: *m/z* 521.04; Calcd. 521.12; Anal. Calcd: C, 69.09; H, 3.87; N, 8.06. Found: C, 69.11; H, 3.87; N, 8.04.

3-(2-(4-(6-chlorobenzo[d]thiazol-2-yl)phenylamino)acetyl)−4−hydroxy-1-phenylquinolin-2(1H)-one (**8b**): Yield 2.60 g (48.41%); R_f 0.68; mp 199–201 °C; IR (KBr, ν, cm^{-1}): 3363.09 (OH Str), 3266.33 (NH Str), 3156.20 (-CH Str), 1708.55 (*C* = *O* Str), 1617.61 (*C* = *N* Str); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 10.63 (s, 1H, OH), 8.56 (s, 1H, NH), 8.14–6.92 (m, 16H, Ar-H), 5.05 (s, 2H, CH_2); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 191.79, 166.64, 166.73, 151.40, 149.52, 138.52, 136.21, 136.01, 129.04, 128.66, 127.20, 125.20, 124.74, 122.71, 122.11, 119.29, 113.63, 113.12, 111.30, 56.30; LCMS: *m/z* 538.92; Calcd. 537.09; Anal. Calcd: C, 66.97; H, 3.75; N, 7.81. Found: C, 67.00; H, 3.76; N, 7.79.

3-(2-(4-(6-bromoobenzo[d]thiazol-2-yl)phenylamino)acetyl)−4−hydroxy-1-phenylquinolin-2(1H)-one (**8c**): Yield 3.10 g (53.35%); R_f 0.36; mp 265–267 °C; IR (KBr, ν, cm^{-1}): 3443.04 (OH Str), 3357.88 (NH Str), 2923.04 (C–H Str), 1711.96 (*C* = *O* Str), 1654.35 (*C* = *O* Str), 1605.49 (*C* = *N* Str); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 10.86 (s, 1H, OH), 8.74 (s, 1H, NH), 8.12–6.58 (m, 16H, Ar-H), 5.57 (s, 2H, CH_2); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 192.55, 165.60, 164.70, 151.34, 149.02, 138.17, 136.22, 136.98, 129.20, 128.61, 126.00, 125.65, 124.29, 122.65, 122.01, 120.01, 114.34, 114.11, 110.29, 57.44; LCMS: *m/z* 580.97; Calcd. 581.04; Anal. Calcd: C, 61.86; H, 3.46; N, 7.21. Found: C, 61.89; H, 3.44; N, 7.21.

3-(2-(4-(benzo[d]thiazol-2-yl)phenylamino)acetyl)−4−hydroxy-1-phenylquinolin-2(1H)-one (**8d**): Yield 3.50 g (69.59%); R_f 0.61; mp 168–170 °C; IR (KBr, ν, cm^{-1}): 3478.43 (OH Str), 3336.81 (NH Str), 2990.30 (-CH Str), 1732.05 (*C* = *O* Str), 1655.33 (*C* = *O* Str), 1614.62 (*C* = *N* Str); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 11.06 (s, 1H, OH), 8.62 (s, 1H, NH), 7.85–7.12 (m, 17H, Ar-H), 5.03 (s, 2H, CH_2); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 191.27, 168.76, 166.48, 153.76, 149.90, 139.29, 136.33, 135.78, 128.78, 128.30, 127.30, 125.09, 124.20, 122.37, 122.10, 119.06, 113.74, 113.25, 109.22, 57.30;

LCMS: *m/z* 503.05; Calcd. 503.13; Anal. Calcd: C, 71.55; H, 4.20; N, 8.34. Found: C, 71.25; H, 4.21; N, 8.36.

3-(2-(4-(7–chloro–6-fluorobenzo[d]thiazol-2-yl)phenylamino)acetyl)–4–hydroxy-1-phenylquinolin-2(1H)-one (**8e**): Yield 3.90 g (70.27%); R_f 0.72; mp 233–235 °C; IR (KBr, ν, cm^{-1}): 3472.76 (OH Str), 3335.23 (NH Str), 3082.20 (-CH Str), 1720.54 (*C = O* Str), 1667.35(*C = O* Str), 1635.44 (*C = N* Str); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 10.81 (s, 1H, OH), 8.71 (s, 1H, NH), 8.18–7.04 (m, 15H, Ar-H), 4.87 (s, 2H, CH_2); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 193.50, 167.88, 165.02, 152.27, 148.41, 139.31, 136.73, 135.65, 128.11, 128.65, 127.11, 125.22, 124.09, 122.45, 122.00, 118.96, 114.54, 114.05, 109.20, 56.72; LCMS: *m/z* 555.05; Calcd. 555.08; Anal. Calcd: C, 64.81; H, 3.44; N, 7.56. Found: C, 64.80; H, 3.43; N, 7.56.

3-(2-(4-(6,7-dichlorobenzo[d]thiazol-2-yl)phenylamino)acetyl)–4–hydroxy-1-phenylquinolin-2(1H)-one (**8f**): Yield 2.75 g (48.16%); R_f 0.69; mp 269–271 °C; IR (KBr, ν, cm^{-1}): 3498.74 (OH Str), 3356.44 (NH Str), 3089.96 (-CH Str), 1716.03 (*C = O* Str), 1661.75(*C = O* Str), 1606.00 (*C = N* Str); ^{1}H NMR (400 MHz, DMSO–d_6, δ, ppm): 10.39 (s, 1H, OH), 8.54 (s, 1H, NH), 8.09–6.73 (m, 15H, Ar-H), 5.16 (s, 2H, CH_2); ^{13}C NMR (400 MHz, DMSO–d_6, δ, ppm): 192.18, 168.58, 166.39, 154.33, 148.01, 139.44, 137.03, 136.03, 128.12, 128.62, 127.12, 125.22, 124.77, 122.39, 122.30, 118.92, 114.90, 114.25, 109.21, 56.74; LCMS: *m/z* 577.32; Calcd. 571.05; Anal. Calcd: C, 62.94; H, 3.35; N, 7.34. Found: C, 62.96; H, 3.36; N, 7.32.

4. Biological evaluation: anticancer activity

4.1. Human cancer cell lines

Compounds were evaluated for their *in vitro* cytotoxic activity against four human cancer cell lines (EAC, MCF-7, HT29, and WRL68 cells), which were cultured in MEM medium supplemented with 10% FBS, 1% L-glutamine, and 50 $mg.mL^{-1}$ gentamicin sulfate in a CO_2 incubator in a humidified atmosphere of 5% CO_2 and 95% air. The EAC cells were maintained for 12–14 days in the peritoneal cavity of Swiss albino mice. The tumor cell cultures were started from mouse Ehrlich Ascites with at least one passage *in vitro* prior to use.

4.1.1. In vitro *cytotoxicity studies (MTT assay)*

In vitro cytotoxicity was determined using a standard MTT assay [36] with protocol appropriate for the individual test system. In brief, exponentially growing cells were plated in 96-well plates (10^4 cells/well in 100 mL of medium) and incubated for 24 h to enable them to adhere properly in the each well of 96-well polystyrene microplate (Grenier, Germany). Test compounds (**7a-f** and **8a-f**) were prepared prior to the experiment by dissolving in 0.1% dimethyl sulfoxide (DMSO, Merck, Germany) and diluted with medium. The cells were then exposed to different concentrations of the drugs (1–100 µM) in the volume of 100 µL per well. Cells in the control wells received the same volume of medium containing 0.1% DMSO. After 24 h incubation, 10 μL of MTT [3-(4,5-dimethylthiazol-2-yl)–2,5-diphenyl tetrazolium bromide; Sigma M 2128; 1 $mg.mL^{-1}$] was added in each well and plates were further incubated at 37 °C for 4 h. The formazan produced by the viable cells was solubilized by addition of 100 µL DMSO. The suspension was placed on micro-vibrator for 5 min and absorbance was recorded by a SpectraMax 190 Microplate ELISA reader (Molecular Devices Inc. USA) at 540 nm. The experiment was performed in triplicate. The percentage cytotoxicity and IC_{50} values were determined at 24, 48, and 72 h of drug incubation. IC_{50} value is the concentration (reported in μM) required for 50% inhibition of cell growth as compared to that of untreated control.

4.1.2. In vitro *antimicrobial activity*

4.1.2.1. Medium. The solid media Mullere Hinton agar (MHA; beef infusion 300 $g.L^{-1}$, casein acid hydrolysate 17.5 $g.L^{-1}$, starch 1.5 $g.L^{-1}$, agar 17 $g.L^{-1}$, and distilled water 1000 mL, adjusted to pH 7.4) was used for the antibacterial activity. For antifungal activity, solidified sterile Sabouraud's Dextrose agar (SDA) was used as medium.

4.1.2.2. Test microorganisms. In vitro antimicrobial activity was carried out against a panel of microorganisms including Gram positive bacteria *Staphylococcus aureus* (ATCC 11,632), *Bacillus subtilis* (ATCC 60,511) and Gram negative bacteria *E. coli* (ATCC 10,536), *Pseudomonas aeruginosa* (ATCC 10,145). For the antifungal activity, the title compounds were screened against *Candida albicans* (ATCC 2501) and *Aspergillus niger* (ATCC 1781).

4.1.2.3. Minimum inhibitory concentration [49]. The *in-vitro* antimicrobial activity for newly synthesized compounds (**7a-f** and **8a-f**) was evaluated using the conventional agar-dilution method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) [50]. All bacterial strains were subcultured in MHA plates and incubated overnight at 37 °C and fungal strains were subcultured in SDA plates at 35 °C for 24–48 h. The microorganisms were processed at least twice to ensure purity and viability. Twofold serial dilutions of the compounds and reference drugs (Ampicilin, Gatifloxacin, and Fluconazole) were prepared in MHA/SDA. Drugs (10.0 mg) were dissolved in DMSO (1 mL) and the solution was diluted with water (9 mL). Further progressive double dilution with melted MHA was performed to obtain the required concentrations of 1024, 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, and 0.0625 $\mu g.mL^{-1}$. The bacterial inoculates were prepared by suspending 24 h old bacterial colonies from MHA media in 0.85% saline. The inoculate were adjusted to 0.5 McFarland Standard (1.5×10^8 CFU/mL) [51]. The suspensions were then diluted in 0.85% saline to give 10^7 CFU/mL. Test tubes were spot-inoculated with 10 µL of each prepared bacterial suspension (10^4 CFU/spot) and incubated at 37 °C for 24 h. The experiments were carried out in triplicate for all the organisms tested. At the end of the incubation period, MIC was determined, which is the lowest concentration of the test compound that resulted in no visible growth on the plate. A control test was also performed with test medium supplemented with DMSO at the same dilutions as used in the experiment in order to ensure that the solvent had no influence on bacterial growth. The yeast suspensions used for inoculation were prepared at 10^4 cfu/mL by diluting fresh cultures at MacFarland 0.5 density (10^6 cfu/mL). Suspensions of the yeast at 10^4 cfu/mL concentration were inoculated to the twofold diluted solution of the compounds. There were 10^3 cfu/mL bacteria in the wells after inoculations. A 10 µL yeast inoculum was added to each well of the test tubes, which were incubated at 35 °C in a humid chamber and MIC endpoints were read after 48 h of incubation. The experiments were carried out in triplicate for all the organisms tested.

4.1.2.4. Docking methodology. Molecular docking studies of the synthesized compounds (**7a-f** and **8a-f**) were performed in order to rationalize the obtained anticancer results especially against hormone dependant breast cancer cells. Molecular docking studies further help in understanding the various interactions between the ligand and enzyme active site in detail. Docking study was carried out for the target compounds using MVD-2013 (version: 6.0). MolDock scoring function used by MVD program is defined by:

$$E_{score} = E_{inter} + E_{intra}$$

where, E_{score} = MolDock score.

E_{inter} = ligand − −Protein interaction

E_{intra} = internal energy of the ligand

MolDock is based on a new heuristic search algorithm that combines differential evolution with a cavity prediction algorithm. The docking scoring function of MolDock is an extension of the piecewise linear potential (PLP) including new hydrogen bonding and electrostatic terms. To further improve docking accuracy, a re-ranking scoring function was introduced, which identifies the most promising docking explanation from the results obtained by the docking algorithm [52].

The compounds/ligands were built using Chemdraw10.0. The 2D structures were then converted into energy minimized 3D structures which were saved as MDL MolFile (.mol2). The coordinate file and crystal structure of EGFR tyrosine kinase (PDB ID: 1M17) was obtained from the RCSB PDB website [47]. The protein file was prepared by the removal of water molecules, addition of polar hydrogens and removal of other bound ligands. In the present study, the binding site was selected based on the amino acid residues, which are involved in binding with Erlotinib (AQ4) of prepared protein as obtained from protein data bank, which would be considered as the probable best accurate region as it is solved by experimental crystallographic data. The docking protocol was carried out for synthesized compounds/ligands as listed in Table 6 using MVD-2013 (6.0) software and the standard operating procedures [30,52].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors state no conflict of interest.

CRediT authorship contribution statement

Girish Bolakatti: Conceptualization, Methodology. **Mahesh Palkar:** Data curation, Writing - original draft. **Manjunatha Katagi:** Visualization, Investigation. **Girish Hampannavar:** Writing - review & editing. **Rajshekhar V. Karpoormath:** Software, Validation. **Shilpa Ninganagouda:** Software, Conceptualization. **Arvind Badiger:** Supervision.

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Supplementary materials

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