Synthesis and \textit{in vitro} anticancer evaluation of some fused indazoles, quinazolines and quinolines as potential EGFR inhibitors

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ABSTRACT

derivatives of benzo[g]indazole \textit{5a, b}, benzo[h]quinazoline \textit{7, 12a-c, 13a-c and 15a-c} and benzo[h]quinoline \textit{17a-c and 19a-c} were synthesized from 6-methoxy-3,4-dihydronaphthalen-1(2H)-one (\textit{1}). Anticancer activity of all the synthesized compounds was evaluated against four cancerous cell lines; HepG2, MCF-7, HCT116 and Caco-2. MCF-7 cells emerged as the most sensitive cell line against the target compounds. All the examined compounds, except \textit{5a} and \textit{5b}, displayed potent to moderate anticancer activity against MCF-7 cells with an IC\textsubscript{50} values ranging from 7.21 to 21.55 µM. In particular, compounds \textit{15c} and \textit{19b} emerged as the most potent derivatives against EGFR-expressing MCF-7 cells with IC\textsubscript{50} values = 7.70 ± 0.39 and 7.21 ± 0.43 µM, respectively. Additionally, both compounds did not display any significant cytotoxicity towards normal BHK-21 fibroblast cells (IC\textsubscript{50} value > 200 µM), thereby providing a good safety profile as anticancer agents. Furthermore, compounds \textit{15c} and \textit{19b} displayed potent inhibitory activity towards EGFR in the sub-micromolar range (IC\textsubscript{50} = 0.13 ± 0.01 and 0.14 ± 0.01 µM, respectively), compared to that of Erlotinib (IC\textsubscript{50} = 0.11 ± 0.01 µM). Docking studies for \textit{15c} and \textit{19b} into EGFR active site was carried out to explore their potential binding modes. Therefore, compounds \textit{15c} and \textit{19b} can be considered as interesting candidates for further development of more potent anticancer agents.

1. Introduction

Cancer remains as a major health problem and a life-threatening disease. It is considered the second leading cause of mortality, after cardiovascular diseases, accounting for about 14.6% of deaths globally [1]. Among women, breast cancer has stood out as the most frequently diagnosed cancer and the second leading cause of cancer-related deaths in women [2]. The conventional chemotherapy displays a pivotal role in the treatment of various types of cancers, nevertheless, there are numerous challenges [3], of which the poor selectivity that causes undesired side effects on normal cells, stands out as the major one [4]. Multiple drug resistance (MDR) [5,6], the ability of neoplastic cells to survive under anaerobic conditions [7] and the incorporation of multiple enzymes at different stages of cancer development [8] are among the other cancer challenges. These entire challenges make the development of more selective, safer and effective therapies for human malignancies an urgent and critical demand.

Epidermal growth factor receptor (EGFR), a receptor tyrosine kinase, displays a vital role regarding the regulation of numerous cellular functions such as cell proliferation, survival, differentiation and migration [9]. EGFR mediates intracellular signaling (intrinsin intracellular protein-tyrosine kinase activity) in response to various extracellular stimuli (endogenous ligand, like epidermal growth factor (EGF) and transforming growth factor α (TGFα)), leading to DNA synthesis and cell growth [10,11]. Mutations which cause EGFR overexpression and activation are associated with wide variety of cancer types as breast cancer, colorectal carcinoma, non-small cell lung cancer, pancreatic cancer and hepatocellular carcinoma [12]. Accordingly, interruption of the signaling pathway of EGFR, either extracellularly by blocking the binding site of EGFR or intracellularly by inhibiting the tyrosine kinase activity, is important in cancer prevention and treatment [13,14]. It has been established that EGFR is one of the most
important targets for development of novel breast cancer therapeutics [15–17].

In the current medical era, quinazoline is one of the most important heterocyclic scaffolds that emerged as a promising privileged scaffold in cancer drug discovery [18–20]. Interestingly, there are many clinically approved quinazoline-based anticancer drugs with potent EGFR-TK inhibitory activity such as Gefitinib (Iressa®) [21], Afatinib (Gilotrif®) [22], Erlotinib (Tarceva®) [23], Icotinib (Conmana®) [24] and Lapatinib (Tykerb®) (Fig. 1) [25].

Furthermore, quinoline is an interesting fused heterocyclic scaffold present in a variety of FDA-approved marketed anticancer drugs [26] such as; Neratinib (Nerlynx®) [27] which is EGFR-TK inhibitor (Fig. 1), Cabozantinib (Cabometyx®) [28], Bosutinib (Bosulif®) [29] and Lenvatinib (Lenvima®) [30], which are protein kinase inhibitors (Fig. 2).

Moreover, indazole is a well-known fused heterocyclic nucleus possessing interesting biological activities [31–33]. Indazole emerged as an attractive scaffold to develop new anticancer agents [34]. The FDA approved drug; Pazopanib (Votrient®) is an example of indazole based multi-targeted receptor tyrosine kinase inhibitor [35] (Fig. 2).

In the light of the previous facts, herein we reported the synthesis of different sets of benzo[g]indazole 5a, b, benzo[h]quinazoline 7, 12a-c, 13a-c and 15a-c, and benzo[h]quinoline 17a-c and 19a-c derivatives. All the previously prepared and novel derivatives were evaluated for their potential anticancer activity against four cancer cell lines; liver carcinoma cell line (HepG2), breast carcinoma cell line (MCF-7), colon carcinoma cell line (HCT116) and colon carcinoma cell line (Caco-2). Furthermore, the most potent counterparts in this study will be assayed for their potential inhibitory activity towards EGFR. Also, molecular docking study was performed to explore the binding mode and possible interactions with EGFR active site.

2. Results and discussion

2.1. Chemistry

The synthetic pathways adopted for the preparation of the intermediates and the target fused indazoles, quinazolines and quinolines were depicted in Schemes 1–3. In Scheme 1, 6-methoxy-3,4-dihydronaphthalen-1(2H)-one (1) was utilized in the synthesis of the 2-acyethyl-6-methoxy-3,4-dihydronaphthalen-1(2H)-one (3) through its reaction with ethyl acetate in sodium ethoxide (Scheme 1). The keto-enol tautomeric structure of compound 3 was established on the basis of its
NMR and IR spectra. The $^1$H NMR spectrum revealed the absence of the signal of the aliphatic hydrogen at C-2 in naphthalene moiety of 1,3-diketone structure of 3 along with the appearance of D$_2$O exchangeable signal of enolic OH at $\delta$ 16.57 ppm. In addition, the $^1$H NMR spectrum revealed the presence of two sets of signals in 3:1 proportions due to the existing of compound 3 in two keto-enol tautomeric structures,

Scheme 1. Synthesis of fused indazoles 5a, b and fused quinazoline 7.

Scheme 2. Synthesis of benzo[h]quinazolines 12a-c, 13a-c and 15a-c.
R – (OH) = CR’ – CO – Me (3A) and R – CO – CR’ = C(OH) – Me (3B) in 3:1 proportions. Also, the $^{13}$C NMR spectrum of compound 3 showed the signal of naphthalene C-2 (SP$^2$ carbon) at δ 104.7 ppm, due to keto-enol tautomeric structure for 3, instead of the SP$^3$ C-2 in 1,3-diketonic structure of 3.

Next, reaction of 2-acetyl-6-methoxy-3,4-dihydronaphthalen-1(2H)-one (3) with hydrazine hydrate (4a) or phenyl hydrazine (4b) gave the corresponding indazoles 5a, b, respectively. Furthermore, treatment of compound 4 (with guanidine hydrochloride (6) in refluxing ethanol, in the presence of potassium hydroxide, yielded the corresponding 8-methoxy-5,6-dihydrobenzo[h]quinazolin-2-amine (7) (Scheme 1).

The structures of the indazoles 5a, b and quinazoline 7 were established on the basis of their spectral data. Their $^{1}H$ NMR spectra showed the singlet signals of methyl and methoxy groups at δ range 2.16–2.26 and 3.72–3.81 ppm, respectively, besides, two multiplet signals of the two methylene protons in δ range 2.55–2.71 and 2.81–2.93 ppm, respectively. While the tautomeric NH of indazole 5a appeared as two D$_2$O-exchangeable singlet signals at 12.19 and 12.64 ppm, protons of the NH$_2$ group of quinazoline 7 appeared as a D$_2$O-exchangeable singlet signal at δ 6.20 ppm. Furthermore, the mass spectrum of 7 showed a peak corresponding to its molecular ion at $m/z$ = 241.26 [M$^+$].

On the other hand, the reaction of compound (1) with aldehydes 8a-c in the presence of sodium hydroxide afforded the corresponding 6-methoxy-2-(arylidene)-3,4-dihydronaphthalen-1(2H)-ones 9a-c, respectively (Scheme 2). The structures of these products were established on the basis of their spectral data. For example, their $^{1}H$ NMR spectra exhibited the singlet signal of =CH– proton in the region δ 7.63–7.75 ppm.

Treatment of 9a-c with urea (10a) or thiourea (10b) in refluxing ethanol, in the presence of potassium hydroxide, afforded dihydrobenzoquinazolines 12a-c and tetrahydrobenzoquinazolines 13a-c, respectively (Scheme 2), in the light of their NMR spectra. $^{1}H$ NMR spectrum of 12b revealed the appearance of the D$_2$O exchangeable signal of NH around δ 11.50 ppm. The $^{1}H$ NMR spectra of tetrahydrobenzoquinazolines 13a-c showed the singlet signal of the aliphatic proton of the chiral C4 in the region δ 4.86–5.20 ppm in addition to the appearance of two D$_2$O exchangeable singlet signals of 2 NH around δ 8.60–8.95 and 9.54–9.64 ppm, respectively. $^{13}$C NMR spectra of tetrahydrobenzoquinazolines 13a, c showed the signal of the SP$^3$ carbon (C4) of the dihydropyrimidine ring of 13a, c around δ 58 ppm.

Interestingly, $^{1}H$ NMR spectra of tetrahydrobenzoquinazolines 13a-c represented four different signals, each signal integrates to one proton of aliphatic protons of C5 and C6 (–CH$_2$–CH$_2$–). The latter findings attributed to the asymmetrical electronic environmental around the chiral C4 in dihydropyrimidine moiety. Compounds 12a-c and 13a-c are assumed to be formed via the initial condensation of urea (10a) or thiourea (10b), respectively, to give the intermediate 11a. The subsequent intramolecular cyclization of 11a through Michael type addition of the terminal NH$_2$ group to the exo-cyclic double bond gave the intermediate 11b. In case of urea (10a), oxidation of 11b via elimination of a hydrogen molecule yielded the final isolated dihydrobenzoquinazolines 12a-c whereas in case of thiourea (10b), the intermediate 11b is converted to its isosteric stable tautomers tetrahydrobenzoquinazolines 13a-c (Scheme 2). In addition, benzo[h]quinazolines 15a-c were prepared by the same method used for the synthesis of compound 7 by using arylidenes 9a-c instead of compound 3 (Scheme 2). The amino group of 15a-c was represented by absorption bands in the region 3416–3477 and 3297–3309 cm$^{-1}$ in their IR spectra and as D$_2$O exchangeable singlet signal in the region δ 6.32–6.37 ppm in their $^{1}H$ NMR spectra, respectively.

Furthermore, the reaction of 6-methoxy-2-(arylidene)-3,4-dihydronaphthalen-1(2H)-ones 9a-c with malononitrile (16) or ethyl cyanoacetate (18), and ammonium acetate in n-butanol, resulted in the formation of 2-amino-8-methoxy-4-(aryl)-5,6-dihydrobenzo[h]quinoline-3-carbonitriles 17a-c and 8-methoxy-4-(aryl)-2-oxo-1,2,5,6-tetrahydrobenzo[h]quinoline-3-carbonitriles 19a-c, respectively (Scheme 3). These compounds were also prepared in a one pot reaction by the treatment of compound 1 with aldehydes 8a-c and malononitrile (16) or ethyl cyanoacetate (18), in the presence of ammonium acetate in n-butanol.

The latter synthesized compounds 17a-c and 19a-c were confirmed on the basis of their elemental analysis and spectral data. The IR spectra of 17a-c revealed the bands of the amino groups at 3437–3500 and 3318–3394 cm$^{-1}$ in addition to absorption bands at 2204–2221 cm$^{-1}$ corresponding to the nitrile functions, respectively. While their $^{1}H$ NMR spectra showed a D$_2$O exchangeable singlet signal of NH$_2$ protons at δ 6.46–6.58 ppm. The IR spectra of 19a-c revealed the appearance of three absorption bands at 3440–3472, 3297–3309 cm$^{-1}$ due to NH, nitrile and carbonyl groups, respectively. While their $^{1}H$ NMR spectra represented the NH proton as a D$_2$O exchangeable singlet signal at δ 12.18–12.49 ppm.

![Scheme 3. Synthesis of benzo[h]quinolines 17a-c and 19a-c.](image-url)
2.2.1. In vitro anti-proliferative activity against HEPG2, MCF-7, HCT116, Caco-2 and BHK-21 cancer cell lines.

The anticancer activity of the synthesized compounds (5a, 5b, 7, 12a-c, 13a-c, 15a-c, 17a-c and 19a-c) was evaluated against four cancerous cell lines; liver carcinoma (HEPG2), breast carcinoma (MCF-7), colon carcinoma (HCT116 and Caco-2) cell lines, using (SRB) colorimetric assay. Dexorubicin and Erlotinib were included in the experiments as reference cytotoxic compounds for all the tested cell lines. The results were expressed as median growth inhibitory concentration (IC50) values, which represent the concentration of a drug that is required for 50% inhibition of cell growth after 48h of incubation, compared to untreated controls (Table 1).

From IC50 values, we can deduce that the synthesized compounds showed good to moderate cytotoxic activity against the tested cancer cell lines. The most active derivative with IC50 value equals 7.84 µM, which was the most active compound throughout this study with IC50 values equal 7.70 µM. Interestingly, MCF-7 cell line emerged as the most sensitive one to the influence of the target compounds as all of the tested compounds except 5a and 5b showed potent to moderate activity with IC50 values ranging from 7.21 to 21.55 µM. In addition, compounds 15c and 19b were the most potent EGFR inhibitors in this study that displayed comparable potency (IC50 = 0.13 ± 0.01 and 0.14 ± 0.01 µM, respectively) to the reference drug Erlotinib (IC50 = 0.11 ± 0.01 µM). In addition, compound 13b displayed potent EGFR inhibitory activity with IC50 value equals 0.49 ± 0.02 µM.

2.2.3. In vitro EGFR kinase assay

The most potent compounds against EGFR-expressing MCF-7 cell line 13a, 13b, 15a, 15c, 17a, 19b, 19c were selected to investigate their potential inhibitory activity towards EGFR by use of an ADP-Glo™ Kinase Assay. The results were reported as 50% inhibition concentration values (IC50) and were summarized in Table 2. The results revealed that the examined compounds exhibited EGFR inhibitory activity with IC50 values ranging from 0.13 to 3.50 µM. Compounds 15c and 19b were the most potent EGFR inhibitors in this study that displayed comparable potency (IC50 = 0.13 ± 0.01 and 0.14 ± 0.01 µM, respectively) to the reference drug Erlotinib (IC50 = 0.11 ± 0.01 µM). In addition, compound 13b displayed potent EGFR inhibitory activity with IC50 value equals 0.49 ± 0.02 µM.

2.3. Molecular docking

Docking of the most potent EGFR-TK inhibitors (15c and 19b) was  

### Table 1
In vitro anti-proliferative activity of the prepared compounds against HEPG2, MCF-7, HCT116, Caco-2 and BHK-21 cancer cell lines.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (µM)( ^a )</th>
<th>HEPG2</th>
<th>MCF-7</th>
<th>HCT116</th>
<th>Caco-2</th>
<th>BHK-21</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>29.40 ± 1.76</td>
<td>NA(^b)</td>
<td>16.33 ± 1.48</td>
<td>NA(^b)</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>5b</td>
<td>NA(^b)</td>
<td>NA(^b)</td>
<td>23.07 ± 1.4</td>
<td>NA(^b)</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>31.08 ± 2.17</td>
<td>21.55 ± 0.93</td>
<td>24.87 ± 1.49</td>
<td>NA(^b)</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>12a</td>
<td>NA(^b)</td>
<td>18.84 ± 1.33</td>
<td>NA(^b)</td>
<td>11.96 ± 0.62</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>12b</td>
<td>NA(^b)</td>
<td>12.35 ± 0.74</td>
<td>NA(^b)</td>
<td>16.19 ± 1.32</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>12c</td>
<td>NA(^b)</td>
<td>14.82 ± 1.36</td>
<td>NA(^b)</td>
<td>23.33 ± 1.20</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>13a</td>
<td>15.60 ± 0.79</td>
<td>11.35 ± 1.09</td>
<td>28.37 ± 1.99</td>
<td>&gt;200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13b</td>
<td>15.69 ± 1.29</td>
<td>13.86 ± 1.11</td>
<td>NA(^b)</td>
<td>20.39 ± 2.04</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>13c</td>
<td>7.84 ± 0.47</td>
<td>19.35 ± 1.94</td>
<td>NA(^b)</td>
<td>21.44 ± 1.32</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>15a</td>
<td>NA(^b)</td>
<td>10.20 ± 0.61</td>
<td>13.50 ± 0.69</td>
<td>NA(^b)</td>
<td>&gt;200</td>
<td></td>
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<tr>
<td>15b</td>
<td>16.51 ± 1.16</td>
<td>17.89 ± 1.26</td>
<td>12.38 ± 1.24</td>
<td>NA(^b)</td>
<td>&gt;200</td>
<td></td>
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<tr>
<td>15c</td>
<td>24.76 ± 1.94</td>
<td>7.70 ± 0.39</td>
<td>23.66 ± 1.69</td>
<td>NA(^b)</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>17a</td>
<td>15.67 ± 0.94</td>
<td>12.03 ± 1.22</td>
<td>17.35 ± 1.42</td>
<td>NA(^b)</td>
<td>&gt;200</td>
<td></td>
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<tr>
<td>17b</td>
<td>18.58 ± 2.42</td>
<td>17.55 ± 1.59</td>
<td>20.39 ± 1.01</td>
<td>NA(^b)</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>17c</td>
<td>13.68 ± 1.24</td>
<td>14.45 ± 0.89</td>
<td>12.91 ± 1.55</td>
<td>NA(^b)</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>19a</td>
<td>NA(^b)</td>
<td>18.14 ± 1.63</td>
<td>NA(^b)</td>
<td>NA(^b)</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>19b</td>
<td>NA(^b)</td>
<td>7.21 ± 0.43</td>
<td>NA(^b)</td>
<td>NA(^b)</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>19c</td>
<td>NA(^b)</td>
<td>13.65 ± 0.96</td>
<td>NA(^b)</td>
<td>NA(^b)</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>Dext.</td>
<td>1.95 ± 0.11</td>
<td>1.10 ± 0.06</td>
<td>0.63 ± 0.03</td>
<td>3.04 ± 0.18</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>Erlotinib</td>
<td>10.19 ± 0.51</td>
<td>5.06 ± 0.27</td>
<td>13.22 ± 0.71</td>
<td>19.13 ± 0.88</td>
<td>&gt;200</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) IC50 values are the mean ± SD of three separate experiments. 
\(^b\) NA: Compounds having IC50 value > 50 µM.

### Table 2
IC50 values of the EGFR inhibitory activity of compounds (13a, 13b, 15a, 15c, 17a, 19b and 19c).

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (µM)( ^a )</th>
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<tbody>
<tr>
<td>13a</td>
<td>1.39 ± 0.08</td>
</tr>
<tr>
<td>13b</td>
<td>0.48 ± 0.02</td>
</tr>
<tr>
<td>15a</td>
<td>3.50 ± 0.18</td>
</tr>
<tr>
<td>15c</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>17a</td>
<td>0.74 ± 0.07</td>
</tr>
<tr>
<td>19b</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td>19c</td>
<td>1.44 ± 0.12</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>0.11 ± 0.01</td>
</tr>
</tbody>
</table>

\(^a\) IC50 values are the mean ± SD of three separate experiments.
carried out to study their pattern of binding and potential binding interactions into the ATP binding site of the EGFR kinase domain. The ability of compounds 15c and 19b to interact with the key amino acids in the ATP binding site of EGFR-TK rationalized their promising anti-tumor activities.

Erlotinib (the co-crystallized ligand for PDB ID: 1M17) showed three hydrogen bonding interactions between N\textsubscript{1} of quinazoline moiety and Met769, a water mediated hydrogen bond between N\textsubscript{3} of quinazoline and Thr766 \cite{36}, and between oxygen atom of the ether side chain and Cys773, in addition to a π-cation interaction between the phenyl ring of 4-anilino moiety and Lys721 (Fig. 3).

Docking simulations for compounds 15c and 19b showed that they fit into the EGFR active site almost at the same manner of Erlotinib with comparable docking scores (−7.97 kcal/mol for Erlotinib, −6.60 and −5.86 kcal/mol for compounds 15c and 19b, respectively).

Compound 15c displayed two essential interactions, as Erlotinib, through two hydrogen bonding between N\textsuperscript{1} and N\textsuperscript{3} of quinazoline moiety, and Met769 and Thr766, respectively. In addition, Methoxy group showed hydrogen bonding interaction with Lys721 and a π-cation interaction with Phe699 (Fig. 4).

Furthermore, compound 19b revealed two hydrogen bonds between the carbonyl group and Met769, and a water mediated between nitrile group and Thr766, in addition to, a π-cation interaction between pyridone ring and Gly772 (Fig. 5).

3. Conclusion
In summary, this study reports the facile synthesis of potent and selective series of benzo[g]indazole (5a, b), benzo[h]quinazoline (7, 12a-c, 13a-c and 15a-c), and benzo[h]quinoline (17a-c and 19a-c) derivatives. All the prepared compounds were examined for their anticancer activity against four cancer cell lines; HepG2, MCF-7, HCT116 and Caco-2. Compounds 15c and 19b showed a promising anticancer activity against MCF-7 cell line as they were the most potent derivatives with IC\textsubscript{50} values = 7.70 ± 0.39 and 7.21 ± 0.43 μM, respectively. Also, compounds 15c and 19b displayed potent inhibitory activity towards EGFR-TK (IC\textsubscript{50} = 0.13 ± 0.01 and 0.14 ± 0.01 μM, respectively). Furthermore, the molecular docking study explored the binding mode and possible interactions between the synthesized compounds and ATP binding site of EGFR kinase domain.

4. Experimental
4.1. Chemistry
4.1.1. General
Melting points were measured with a Stuart melting point apparatus and were uncorrected. Infrared spectra were recorded on Shimadzu FT-IR spectrophotometer as potassium bromide discs. Mass spectra (MS) were performed at 70 eV by GCMS-QP1000 EX spectrometer using the
400MHz. 1H spectra were run at 300 or 400MHz, while 13C spectra were run at 75MHz in deuterated dimethyl sulfoxide (DMSO-d6). Chemical shifts are expressed in values (ppm) using the solvent peak as internal standard. All coupling constant (J) values are given in hertz. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet. Elemental analyses were carried out at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. Analytical thin layer chromatography (TLC) on silica gel plates containing UV indicator was employed routinely to follow the course of reactions and to check the purity of products. All reagents and solvents were purified and dried by standard techniques.

4.1.2. Synthesis of 2-acetyl-6-methoxy-3,4-dihydronaphthalen-1(2H)-one 3

6-Methoxy-3,4-dihydronaphthalen-1(2H)-one (3) (1.76 g, 10 mmol) was added to a sodium ethoxide solution prepared from sodium metal (0.46 g, 20 mmol) and absolute ethanol (20 mL). After stirring for 15 min, ethyl acetate (2) (1.62 g, 20 mmol) was added and the mixture was refluxed for 4 h. After cooling, the solution mixture was poured onto cold water; neutralized with acetic acid (10%) and left in the refrigerator overnight. The solid product was collected by filtration, washed with diethyl ether, dried and finally crystallized from ethanol to yield compounds 3 as white powder in 79% yield, m.p. 114–116°C (re-reflux for 24 h. The produced product was filtered, washed with ethanol, dried and crystallized from EtOH/DMF to give compound 3 as grey powder in 84% yield, m.p. 139.5°C [38]; IR (KBr, ν cm⁻¹): 3174 (OH); 1H NMR (DMSO-d6, 400 MHz) δ ppm: 2.18, 2.25 (s, 3H, CH3), 2.56–2.60 (m, 2H, CH2), 2.81–2.96 (m, 2H, CH2, 3.83 (s, 3H, OCH3), 6.89–6.93 (m, 2H, H-5 and H-7), 7.78, 7.83 (d, J = 8.4 Hz, 1H, H-8); 16.57 (s, 1H, OH, D2O exchangeable); 13C NMR (DMSO-d6, 75 MHz) δ ppm: 22.0 and 22.5 (tautomeric C3), 25.0 (CH3), 27.3 and 27.7 (tautomeric C4), 55.3 (OCH3), 104.7 (C2), 112.6, 113.4, 123.5, 127.5, 143.7, 162.5, 178.5, 190.0 (C=O); MS (EI) m/z (%): 218.04 (M +, 19.2), 63.99 (100); Anal. Calcd. for C14H15N3O (241.29): C, 72.87; H, 6.59; N, 17.41; Found C, 72.80; H, 6.59; N, 17.30.

4.1.3. Synthesis of benzo[g]indazoles 5a,b

4.1.3.1. 7-Methoxy-3-methyl-4,5-dihydro-2H-benzof[g]indazole (5a).

To a solution of 2-acetyl-6-methoxy-3,4-dihydronaphthalen-1(2H)-one 3 (0.218 g, 1 mmol) in absolute ethanol (50 mL), hydrazine hydrate (0.218 g, 1 mmol) in glacial acetic acid (50 mL), phenyl hydrazine (4b) (0.11 g, 1 mmol) was added. The reaction mixture was refluxed for 5 h, then left to cool and pour onto ice. The formed precipitate was filtered and crystallized from ethanol to give indazole 5b as buff powder in 84% yield, m.p. 135–137°C; IR (KBr, ν cm⁻¹): 3174 (OH); 1H NMR (DMSO-d6, 400 MHz) δ ppm: 2.19 (s, 3H, CH3), 2.57–2.60 (m, 2H, CH2), 2.90–2.93 (m, 2H, CH2), 3.72 (s, 3H, OCH3), 6.61 (s, 2H, Ar H), 6.95 (s, 1H, Ar H), 7.41–7.53 (m, 5H, Ar H); 13C NMR (DMSO-d6, 75 MHz) δ ppm: 11.2 (CH3), 18.5 (C4), 30.1 (C5), 54.9 (OCH3), 111.2, 111.2, 114.5 (2C), 116.6, 119.4, 123.3, 125.2, 127.7, 129.1, 137.2, 138.8, 140.5, 144.9, 158.3; MS (EI) m/z (%): 290.21 (M⁺, 67.3), 57.11 (100); Anal. Calcd. for C15H14N3O2 (290.36): C, 73.79; H, 6.25; N, 9.65; Found C, 73.74; H, 6.21; N, 9.59.

4.1.4. 8-Methoxy-4-methyl-5,6-dihydrobenzo[h]quinazolin-2-amine (7)

To a solution of 2-acetyl-6-methoxy-3,4-dihydronaphthalen-1(2H)-one 3 (0.218 g, 1 mmol) and guanidine hydrochloride (6) (0.19 g, 2 mmol) in absolute ethanol (30 mL), potassium hydroxide (0.17 g, 3 mmol) was added and the mixture was heated under reflux for 24 h. The produced product was filtered, washed with ethanol, dried and crystallized from EtOH/DMF to give compound 7 as grey powder in 82% yield, m.p. >300°C; IR (KBr, ν cm⁻¹): 3174 and 3298 (NH2), 1632 (C=O); 1H NMR (DMSO-d6, 400 MHz) δ ppm: 2.26 (s, 3H, CH3), 2.68–2.71 (m, 2H, CH2), 2.81–2.85 (m, 2H, CH2), 3.81 (s, 3H, OCH3), 6.20 (s, 2H, NH2, D2O exchangeable), 6.86–6.91 (m, 2H, H-7 and H-9), 8.04 (d, J = 8.4 Hz, 1H, H-10); MS (EI) m/z (%): 241.26 (M⁺, 31.5), 57.09 (100); Anal. Calcd. for C13H11N2O (241.29): C, 69.69; H, 6.27; N, 17.41; Found C, 69.74; H, 6.32; N, 17.52.

4.1.5. General procedure for the synthesis of 6-methoxy-2-(arylidene)-3,4-dihydronaphthalen-1(2H)-ones 9a-c

A mixture of 6-methoxy-3,4-dihydronaphthalen-1(2H)-one 1 (1.76 g, 10 mmol), the appropriate aldehyde 8a-c (10 mmol) and 10% sodium hydroxide solution (10%, 15 mL) in ethanol (30 mL) was stirred for 12 h at room temperature. The separated precipitate was filtered, washed with water and crystallized from ethanol to yield compounds 9a-c, respectively.

4.1.5.1. 6-Methoxy-2-(4-methoxybenzylidene)-3,4-dihydronaphthalen-1(2H)-one 9a.

White powder in 85% yield, m.p. 141–142°C (reported m.p. 139.5°C) [38]; IR (KBr, ν cm⁻¹): 1663 (C=O); 1H NMR (DMSO-d6, 300 MHz) δ ppm: 2.88–2.92 (m, 2H, CH2), 3.04–3.08 (m, 2H, CH2), 3.80 (s, 3H, OCH3), 3.84 (s, 3H, OCH3), 6.88 (d,
J = 2.4 Hz, 1H, H-5), 6.93 (dd, J = 8.7, 2.4 Hz, 1H, H-7), 7.02 (d, J = 8.7 Hz, 2H, H-3’ and H-5’), 7.48 (d, J = 8.7 Hz, 2H, H-2’ and H-6’), 7.63 (s, 1H, –C≡H), 7.92 (d, J = 8.7 Hz, 1H, H-8); 13C NMR (DMSO-\(d_6\), 75 MHz) \(\delta\) ppm: 26.6 (C3), 28.1 (C4), 55.1 (OCH3), 55.4 (OCH3), 112.2, 113.4, 114.0 (2C), 126.4, 127.7, 129.7, 131.5 (2C), 133.4, 134.8, 145.6, 159.5, 163.0, 185.2 \((\text{O=C})\); MS (EI) \(m/z\) (%): 234.45 \((M^+\), 37.8); MS (EI) \(m/z\) (%): 234.33 \((M^+\), 13.5), 45.08 (100); Anal. Calcd. for \(C_{20}H_{20}O_4\) (352.45): C, 68.16; H, 5.72; N, 7.95; Found C, 68.03; H, 5.68; N, 7.88.

4.1.6.5. 2-(4-Dimethylamino)-benzo[h]quinazoline-2(1H)-thione (13a). White powder in 45% yield, m.p. 160–162°C; IR (KBr, \(\nu\) cm\(^{-1}\)): 3194 (2NH), 1250 (C=O); 1H NMR (DMSO-\(d_6\), 400 MHz) \(\delta\) ppm: 7.89–8.03 (2H, H-5 and H-6), 7.65 (d, J = 8.4 Hz, 2H, H-2’ and H-6’), 7.27 (d, J = 8.4 Hz, 2H, H-3’ and H-5’), 6.75 (s, 1H, –C≡H), 7.04 (d, J = 8.4 Hz, 1H, H-7), 7.02 (d, J = 8.4 Hz, 1H, H-8). 15C NMR (DMSO-\(d_6\), 75 MHz) \(\delta\) ppm: 23.5 (C9), 27.7 (C6), 55.0 (OCH3), 57.0 (C8), 104.9, 112.6, 113.4, 116.6, 123.9, 126.5, 129.7, 130.6, 133.3, 145.7, 159.2, 161.4, 162.7, 185.3 \((\text{O=C})\); MS (EI) \(m/z\) (%): 235.32 \((M^+\), 13.3), 45.08 (100); Anal. Calcd. for \(C_{28}H_{22}N_2O_4\) (352.45): C, 68.16; H, 5.72; N, 7.95; Found C, 68.03; H, 5.68; N, 7.88.

4.1.6.6. 2-(4-Dimethylamino)-6-methylbenzo[h]quinazolin-2(1H)-thione (13b). White powder in 59% yield, m.p. 253–255°C (reported m.p. 253–255°C) \([41]\); IR (KBr, \(\nu\) cm\(^{-1}\)): 3186 (2NH), 1257 (C=O); 1H NMR (DMSO-\(d_6\), 400 MHz) \(\delta\) ppm: 7.89–8.03 (2H, H-5 and H-6), 7.65 (d, J = 8.4 Hz, 2H, H-2’ and H-6’), 7.27 (d, J = 8.4 Hz, 1H, H-7), 7.02 (d, J = 8.4 Hz, 1H, H-8). 15C NMR (DMSO-\(d_6\), 75 MHz) \(\delta\) ppm: 23.5 (C9), 27.7 (C6), 55.0 (OCH3), 55.5 (OCH3), 58.1 (C4), 108.5, 110.7, 111.1, 112.0, 113.8, 119.0, 120.6, 122.8, 126.4, 135.4, 137.4, 148.5, 148.7, 158.8, 174.0 \((\text{C=O})\); MS (EI) \(m/z\) (%): 382.17 \((M^+\), 26.18), 40.18 (100); Anal. Calcd. for \(C_{38}H_{28}N_2O_8\) (382.48): C, 65.95; H, 5.80; N, 7.32; Found C, 65.99; H, 5.84; N, 7.36.
4.1.7. General procedure for the synthesis of benzo[h]quinazolines 15a-c
These compounds were prepared by the same method for synthesis of compound 7 by using the appropriate 6-methoxy-2-(arylidene)-3,4-dihydronaphthalen-1(2H)-ones 9a-c instead of 2-acetyl-6-methoxy-3,4-dihydronaphthalen-1(2H)-one (3).

4.1.7.1. 8-Methoxy-4-(4-methoxyphenyl)-5, 6-dihydrobenzo[h]quinazolin-2-amine (15a).
Green powder in 57% yield, m.p. 152–154°C; IR (KBr, ν cm⁻¹): 3436, 3305 (NH), 1608 (C=O); 1H NMR (DMSO-δ6, 400 MHz) δ ppm: 2.33, 2.51 (2s, 2H, CH2), 2.71 (2s, 2H, CH2), 3.47 (2H, OCH3), 3.62 (2H, NH2), 6.60–6.65 (2H, H-10 and H-13), 6.85 (1H, H-7).

4.1.7.2. 4-(2,4-Dimethoxyphenyl)-8-methoxy-5,6-dihydrobenzo[h]quinazolin-2-amine (15b).
Green powder in 70% yield, m.p. 206°C; IR (KBr, ν cm⁻¹): 3416, 3309 (NH), 1605 (C=O); 1H NMR (DMSO-δ6, 400 MHz) δ ppm: 2.75–2.79 (4H, 2CH2), 3.79 (3s, 9H, OCH3), 3.81 (6H, 2OCH3), 6.36 (2H, NH2, D2O exchangeable), 6.86 (1H, H-7), 6.92 (δ, J = 8.9 Hz, H-9), 7.16 (1H, H-7), 8.10 (δ, J = 9.2 Hz, H-10), 11.30 (1H, NH, D2O exchangeable), 1H NMR (DMSO-δ6, 125 MHz) δ ppm: 33.35 (M+, 100); Anal. Calcd. for C22H19N3O2: C, 73.93; H, 5.36; N, 14.71.

4.1.7.3. 4-(3,4-Dimethoxyphenyl)-8-methoxy-5,6-dihydrobenzo[h]quinazolin-2-amine (15c).
Green powder in 68% yield, m.p. 207°C; IR (KBr, ν cm⁻¹): 3416, 3309 (NH), 1605 (C=O); 1H NMR (DMSO-δ6, 400 MHz) δ ppm: 2.75–2.79 (4H, 2CH2), 3.79 (3s, 9H, OCH3), 3.81 (6H, 2OCH3), 6.36 (2H, NH2, D2O exchangeable), 6.86 (1H, H-7), 6.92 (δ, J = 8.9 Hz, H-9), 7.16 (1H, H-7), 8.10 (δ, J = 9.2 Hz, H-10), 11.30 (1H, NH, D2O exchangeable), 1H NMR (DMSO-δ6, 125 MHz) δ ppm: 33.35 (M+, 100); Anal. Calcd. for C22H19N3O2: C, 73.93; H, 5.36; N, 14.71.
calculated. The x-ray crystallographic structure of EGFR kinase domain with MMFF94x force field and the partial charges were automatically used for the docking simulation and calculation. All minimizations were done using Protonate 3D protocol in MOE with default options. Docking was done using Tripos Matchener placement method and London dG scoring function, refinement of the results was done using Force field energy and validation process was carried out by redocking the co-crystallized ligand (erlotinib) into EGFR-TK binding site and then its scoring energy (S) and root mean square deviation (rmsd) were calculated. Finally, its interaction with amino acids in the active site was studied. Docking was done using pharmacophore query having two features (hydrogen bond with Met769 and a water mediated hydrogen bond with Thr766) [36].

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