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Discovery of novel quinazolinones and their acyclic analogues as multi-kinase inhibitors: design, synthesis, SAR analysis and biological evaluation

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This work deals with the design and synthesis of some novel 6-iodo-2-(pyridin-3/4-yl)-3-substituted quinazolin-4-one derivatives **8a–l**, **10a–h**, **13–18** in addition to certain acyclic analogues thereof *viz.* **9a–n** and **12a–h**. The molecular design strategy was based on structural analogy between the new compounds and reported quinazolines and their acyclic analogues. This design scheme led to the synthesis of 8 new intermediates and 58 new final quinazolinones. The target compounds were evaluated for their antitumor activity against a panel of nine cancer cell lines *viz.* breast cancer (MCF-7, MDAMB-231, MDAMB-435 and HS-578T), colon cancer (HT-29 and HCC-2998) and leukemia (CCRF-CEM, K-562 and HL-60). The quinazolinones **10a–h** displayed exceptional antitumor activity and compounds **12a–h** showed superior potency against MCF-7. These compounds were further subjected to *in vivo* study. Kinase inhibitory assay was also carried out to investigate the mechanism of action of the target compounds and they displayed the highest activity against ABL, ALK and c-RAF kinases. The 3-substituted quinazolinones **10a–h** showed the highest kinase activity inhibitory potency against ABL, ALK and c-RAF with the most active compound in this study being the fluoro-3-pyridyl derivative **10a**. These results are in compliance with the observed antitumor activity. Finally, a molecular modeling study was performed to interpret the potential molecular interactions of these chemotypes with the most responsive biomolecular target ABL.

Introduction

Quinazoline nucleus-based small molecules have aroused recent attention from chemical and biological view-points. This is attributed to the fact that members of this chemotype have found their way to the pharmaceutical products market after being granted FDA approval through displaying remarkable antineoplastic activity supported by a diverse array of mechanisms of action.[1–5] Among the disclosed modes of cytotoxicity activities elicited by quinazolines is their effect as dihydrofolate reductase antimetabolites,[6,7] DNA intercalators[3] or protein kinase inhibitors.[8–11]

The human genome contains about 500 protein kinase genes constituting about 2% of all human genes.[12] Up to 30% of all human proteins are modifiable by kinase activity where kinases are well-known regulators of many cellular pathways, especially those involved in signal transduction.[13] Hence, it was found that protein kinases' mutation and/or over-expression play a central role in disruption of various cellular activities like growth, proliferation and migration, and thus is a hallmark of some types of cancer.[12]

Much attention has been given to exploiting small molecules that can target protein kinases as inhibitors where such chemical entities have proved effective as anti-proliferative and/or cytotoxic agents.[10,12,14–16] Quinazoline-based molecules are one such class that act through interfering with the enzymatic phosphorylation ability of kinase enzyme family members. The antikinase activity of quinazolines is generally due to their ability to competitively occupy the adenosine triphosphate (ATP)-binding pocket with high affinity, thus acting as ATP-mimic inhibitors.[17–20]

Despite extensive efforts within the oncology field to develop kinase inhibitors (KIs), uncertainty remains over the relative merits of selective compounds *versus* less selective or "multi-targeted" inhibitors.[21] Targeted molecules offer the clearest indication that *in vivo* effects result from the intended *in vitro* activity. Moreover, toxicity derived from additional activity against other kinases is likely to be reduced.[22] However, inhibition of a single kinase may not be sufficient to achieve a clinical benefit, either through the built-in redundancy of signaling pathways, or the ability of tumors to acquire resistance.[23] Inhibitors with activity against multiple kinases may in

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fact be more effective anticancer agents, and several multi-targeted kinase inhibitors are now commercially available like sorafenib.[24]

Consequently, and motivated by the aforementioned facts, it deemed of interest to design, synthesize and evaluate novel quinazolinones as potential kinase inhibitors and cytotoxic agents.

Molecular design strategy

Design of the target compounds (Fig. 1) focused on alternating the physicochemical properties of the functional groups on the 2,3,6-trisubstituted quinazolinone backbone to target different regions of the ATP-binding pocket of the protein kinase domain. The ATP binding site has multiple regions *viz.* the adenine binding region which contains two key hydrogen bonds formed by the interaction of N-1 and N-6 amino group of the adenine ring; the sugar binding region which is mostly hydrophilic in nature; a hydrophobic pocket that is not used by ATP but plays a crucial role in inhibitor selectivity; hydrophobic channels that are not occupied by ATP yet may be exploited for inhibitor specificity, and finally the phosphate binding region which can be targeted for improving inhibitor selectivity.[20,25]

Molecular manipulations on the quinazoline backbone were performed according to the following rationale:

First the 2-position of the quinazolinone ring, a heteroaryl *viz.* 3-pyridyl or 4-pyridyl was introduced to study the effect of this positional isomerism on the enzymatic and anticancer activities of these derivatives.

For the 3-position, fragment molecular manipulation was carried out with different groups of diverse lipophilicity and length *viz.* substituted/unsubstituted urea or thiourea, carboxamide, carbothioamide, carbothiohydrazide, phenyl hydrazine, hydroxylamine, *para*-aminobenzoic acid, benzylideneamino and substituted acetylhydrazinyl to study the effect of these changes on their occupation of the lipophilic pocket of the ATP binding site of kinases and hence their associated cytotoxic activity as compared to well-known KIs.

Moreover, the 6-position of the quinazolinone was kept occupied by the bulky hydrophobic iodo substituent aiming to furnish the necessary bulkiness and hydrophobic character required at this position besides acting as a metabolic blocker to prolong the duration of action of these molecules.

Finally, modifications related to decreasing the rigidity of the structure through quinazoline ring opening were performed to assess the effect of increasing the backbone flexibility on the molecular binding interactions mode with the active site amino acids of the target enzyme and on hence on the investigated biological activity.

The newly synthesized compounds were initially screened for their cytotoxic activities against a panel of cancer cell lines. Compounds showing remarkable anticancer activity were subjected to *in vivo* study. The kinase inhibitory activity was also evaluated to elucidate the mechanism of action of the active derivatives. Furthermore, a molecular docking study was carried out to predict the plausible binding mode of these chemotypes with the active site of the most responsive kinase ABL.

Chemistry

The key starting materials, 5-iodoanthranilic acid **3**,[26] nicotinoyl chloride **4a** [27] and isonicotinoyl chloride **4b**,[28] were prepared as reported. 5-Iodo-2-[(pyridinyl-3/4-carbonyl)amino]benzoic acid derivatives **5a**, **b** were prepared *via* the reaction of 5-iodoanthranilic acid **3** and the freshly prepared nicotinoyl chloride **4a** or isonicotinoyl chloride **4b** in dry DMF at 80 °C. The benzoxazinone derivatives **6a**, **b** were synthesized through the nucleophilic reaction of the amidated anthranilic acid with acetic anhydride or *via* the reaction of the substituted anthranilic acid and the freshly prepared acyl chloride in dry pyridine at 5 °C. Moreover, the reaction of benzoxazinones **6a**, **b** with hydrazine hydrate was carried out in various solvents or under various reactions conditions *viz.* reflux in ethanol or *n*-butanol or direct solvent-free fusion of the reactants to obtain the desired 3-aminoquinazolinone. In all cases, the reaction afforded the

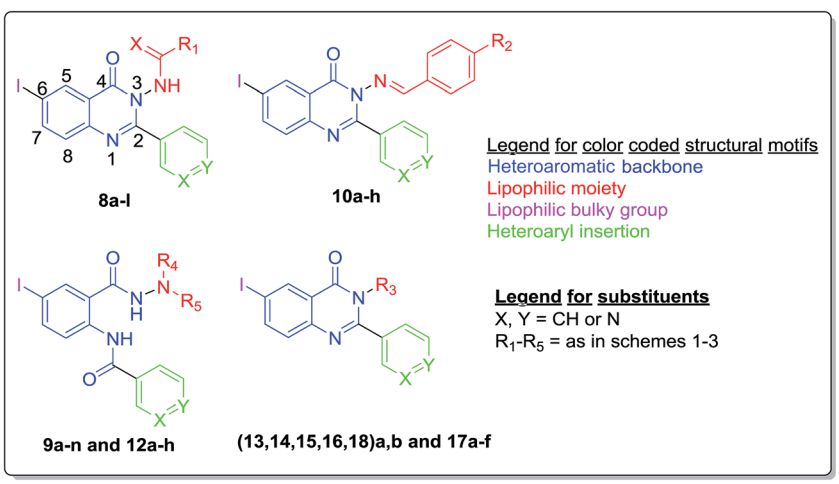
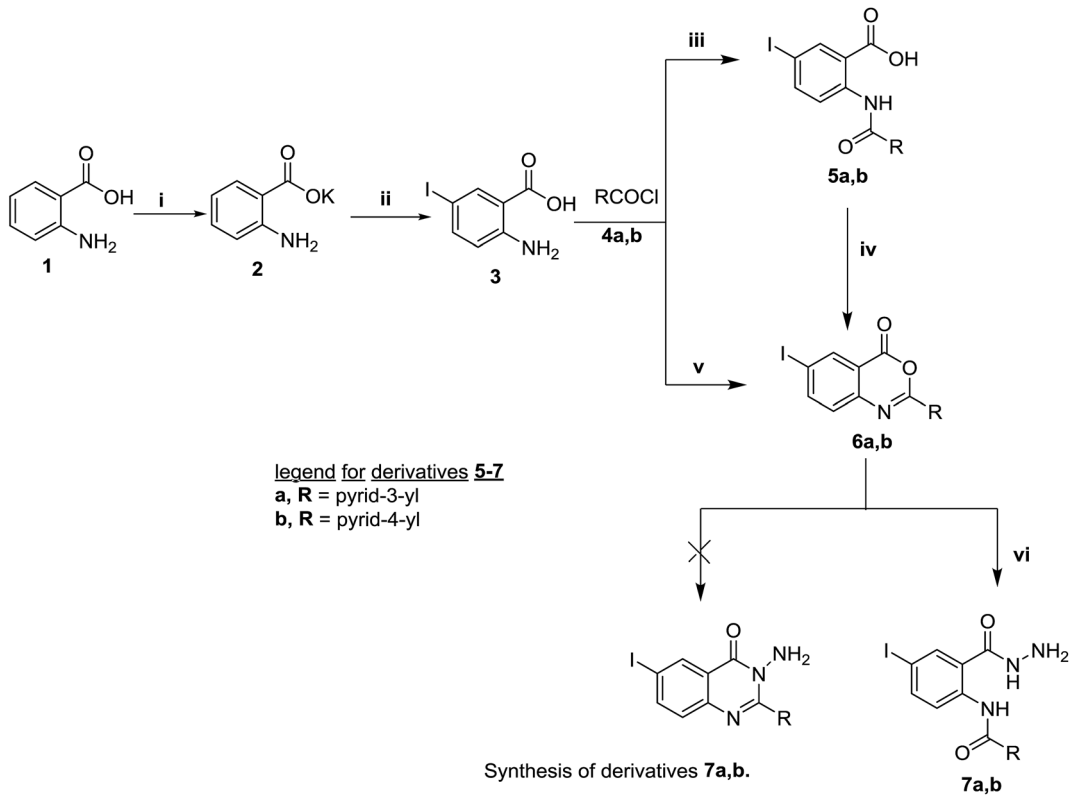


Fig. 1 Design strategy for the target 2,3,6-trisubstituted quinazolinones and their acyclic analogs.



Scheme 1 Synthesis of derivatives **7a**, **b**. Reagents and solvents: (i) KOH; (ii) I_2 KOH, glacial acetic acid; (iii) DMF; (iv) acetic anhydride; (v) dry pyridine; (vi) $NH_2NH_2 \cdot H_2O$, absolute ethanol.

open ring structures; *N*-[2-(hydrazinylcarbonyl)-4-iodophenyl] pyridine-3/4-carboxamide **7a**, **b** which were isolated and identified. Attempts to cyclize the diamides **7a**, **b** to the corresponding 4-(3*H*)-quinazolin-4-one under various reaction conditions, including sodium ethoxide or polyphosphoric acid were unsuccessful (Scheme 1).

Compounds **8a–l** were prepared by refluxing the diamide derivatives **7a**, **b** with the appropriate isocyanate or isothiocyanate in dioxane in the presence of catalytic amount of triethylamine. Synthesis of the benzylideneamino derivatives **9a–n** was carried out by treating the hydrazido derivatives with the appropriate aldehyde in absolute ethanol in presence of few drops of glacial acetic acid. Cyclodehydration reaction was then carried out using sodium ethoxide to obtain the benzylidene aminoquinazolinones **10a–h** from the diamide analogues. Also, the desired 2-chloroacetyl hydrazinyl derivatives **11a**, **b** were successfully synthesized by reacting compounds **7a**, **b** with chloroacetyl chloride in dry dimethylformamide at room temperature. The chloroacetylhydrazinyl derivative**11a** and its 4-pyridyl analogue **11b** were refluxed with morpholine, piperidine or substituted piperazines in absolute ethanol containing anhydrous potassium carbonate to produce the desired compounds **12a–h** (Scheme 2).

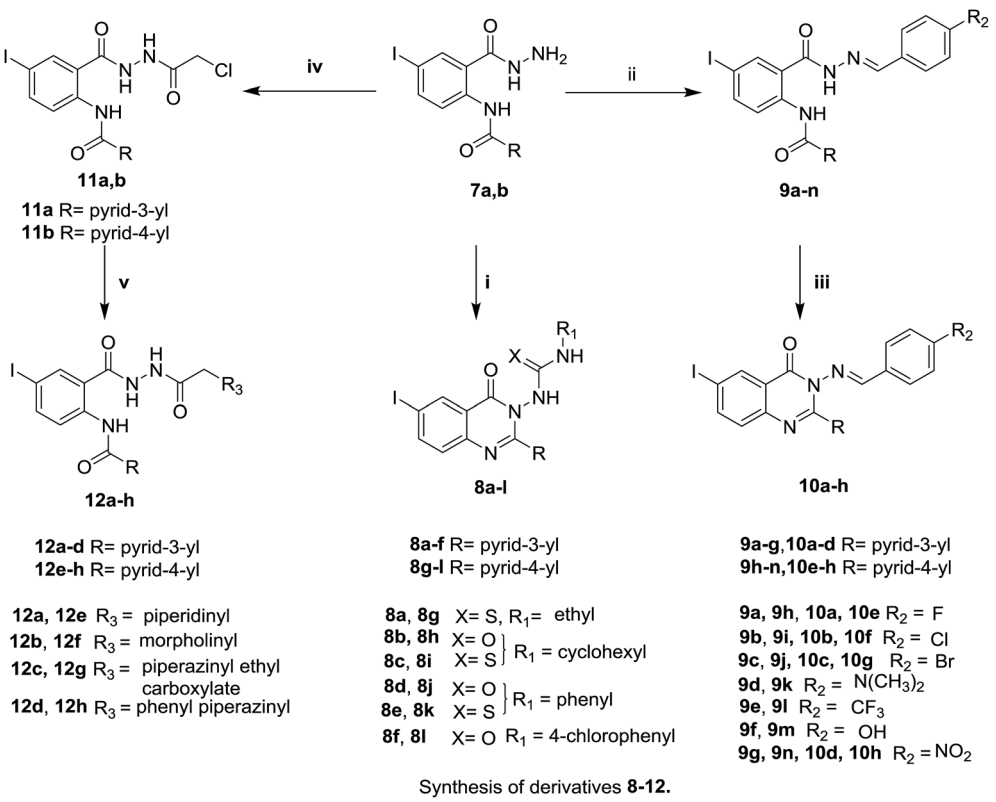
The hydroxy derivatives **13a** and **13b** were synthesized *via* refluxing **6a** and **6b** with hydroxylamine hydrochloride in dry pyridine. Refluxing the benzoxazinone derivatives **6a**, **b** with urea, thiourea or thiosemicarbazide in glacial acetic acid in the presence of anhydrous sodium acetate furnished the carboxamide derivatives **14a**, **b** carbothioamides **15a**, **b** and carbothiohydrazides **16a**, **b**. Moreover, the phenylamino derivatives **17a–f** were prepared through refluxing the various hydrazine derivatives with the benzoxazinone compounds **6a**, **b** in absolute ethanol. Finally, reaction of a mixture of benzoxazinones **6a**, **b** with *p*-aminobenzoic acid under fusion conditions at 200 °C yielded the benzoic acid derivatives **18a**, **b** (Scheme 3).

Results and discussion

(1) Biological evaluation

(a) *In vitro* cytotoxic activity. All the newly synthesized compounds were tested *in vitro* for their cytotoxic activity against a panel of 9 human cancer cell lines. This panel involved four breast cancer cell lines (MCF-7, MDAMB-231, MDAMB-435 and HS-578T), two colon cancer cell lines (HT-29 and HCC-2998) and three leukemia cell lines (CCRF-CEM, K-562 and HL-60). The obtained IC_{50} values are displayed in Table 1.

As shown in Table 1, most of the novel quinazolinones; **10a–h**, **13a**, **b**, **14a**, **b**, **15a**, **b**, **16a**, **b**, **18a**, **b** and **17a–f** and *N*-(2,4-disubstituted phenyl)pyridine-carboxamides; **9a–n** and **12a–h** displayed single digit to sub-nanomolar level cytotoxic activity against the breast cancer cell lines MCF-7, MDA-MB-231, HS-578T and MDA-MB-435; the colon cancer cell lines HT29 and HCC-2998 and leukemia cell lines CCRF-CEM, K-562 and HL-60.

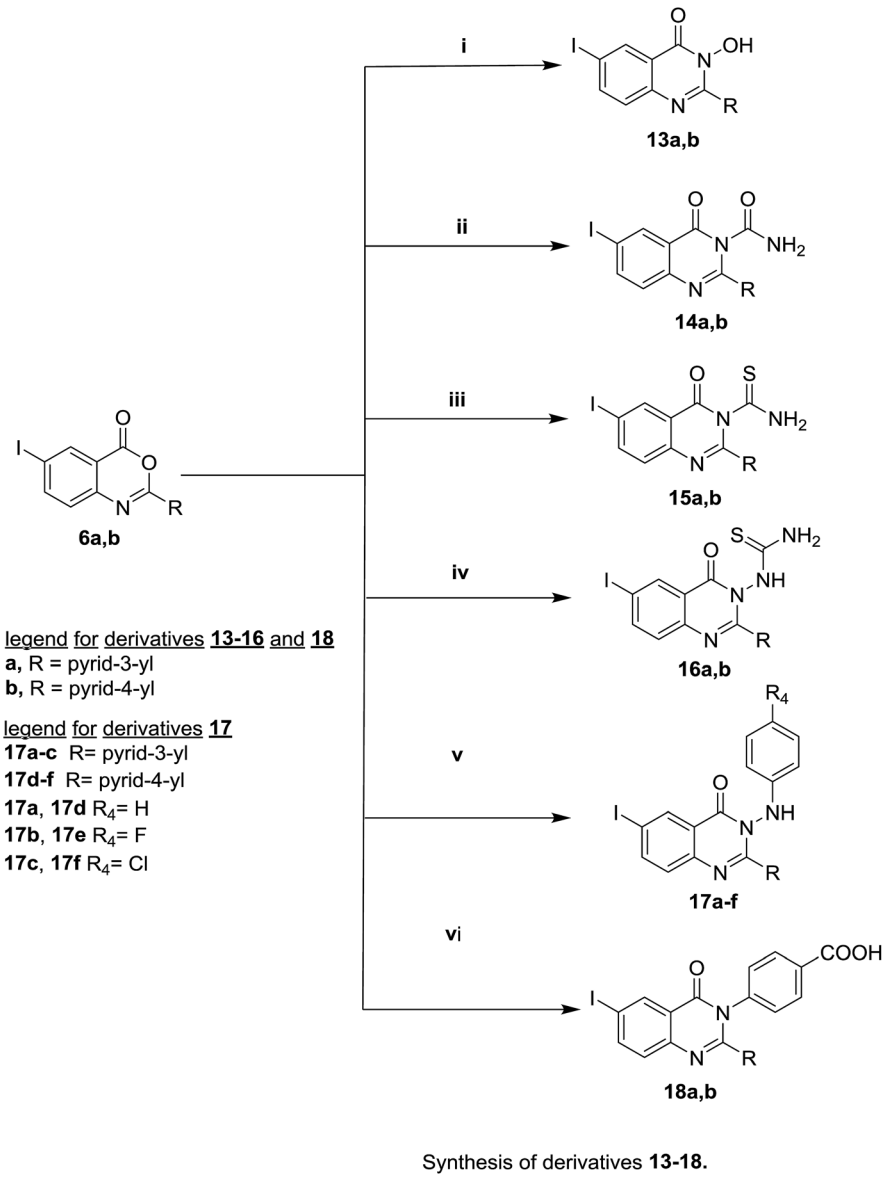


Scheme 2 Synthesis of derivatives **8–12**. Reagents and solvents: (i) appropriate isocyanate/isothiocyanate, TEA, dioxane; (ii) *p*-substituted benzaldehyde, absolute ethanol, glacial acetic acid; (iii) sodium ethoxide, absolute ethanol; (iv) $ClCH_2COCl$, DMF; (v) secondary amines, absolute ethanol, anhydrous K_2CO_3.

SAR analysis into the findings of the cytotoxicity of the synthesized compounds against breast cancer cell lines revealed that most of the quinazolinyl urea/thiourea derivatives **8a–l** had weak activity on MCF-7 (>100 μM). However, most of the pyridinecarboxamides bearing benzylidene moiety **9a–n** exhibited potent growth inhibitory activity. All the 3-pyridyl analogues displayed enhanced activity against all breast cancer cell lines (IC_{50} = 0.0032–0.009 μM). Looking at the 4-pyridyl series, some congeners exhibited weak activity (>100 μM), particularly; the fluoro derivative **9h** against MCF-7, the chloro derivative **9i** against HS-578T and MDA-MB-435, the alkylated and nitro analogues **9l**, **9m** and **9n** against MCF-7 and MDA-MB-435. Interestingly, the target quinazolinones **10a–h**, **13–16, 18a,b** & **17a–f** and pyridinecarboxamides with acetyl hydrazinyl-carrying side chain**12a–h** displayed superior cytotoxic activity against all the breast cancer cell lines. Compounds **12a–h** showed high potency, particularly against MCF-7 (IC_{50} = 0.12–0.78 nM). Furthermore, the quinazolinones Schiff's base derivatives **10a–h** displayed exceptional antitumor activity (IC_{50} = 0.010–0.022 nM) against MCF-7 in the subnanomolar range. Regarding the cytotoxicity against colon HT29 and HCC-2998 cancer cell lines the quinazolinones Schiff's base derivatives **10a–h** (IC_{50} = 0.0031–0.049 μM) and most of the quinazolinyl urea/thiourea derivatives **8a–l** (IC_{50} = 0.0034–0.65 μM) displayed superior anticancer activity. Moreover, the target quinazolinones **13–16, 18a, b** & **17a–f** (IC_{50} = 0.014–0.098 μM) and pyridinecarboxamides with acetyl hydrazinyl-carrying side chain**12a–h** (IC_{50} = 0.017–0.65 μM) displayed high cytotoxicity against the colon cancer cell lines. The pyridinecarboxamides bearing benzylidene moiety **9a–n** exhibited the weakest antineoplastic activity against colon cancer cell lines (IC_{50} = 0.05–0.96 μM). Concerning leukemia CCRF-CEM, K-562 and HL-60 cell lines, all the tested compounds exhibited high growth inhibitory activity (IC_{50} = 0.085–0.0013 μM).

(b) *In vitro* cytotoxicity against the non-tumorigenic MCF-10A cell line and selectivity. Before progressing into the *in vivo* cytotoxic screening against MCF-7 mouse xenograft model, selected compounds *viz.* **10a–h**, **12b** and **12f** were subjected to *in vitro* evaluation against the non-tumorigenic breast cell lines MCF-10A. The cell line used was selected to represent healthy cells and to determine whether the cytotoxic properties of the tested compounds were selective for malignant cells in contrast to nonmalignant cells. Data obtained are enlisted in Table 2.

With regards to selective cytotoxicity of the tested compounds relative to examined breast cancer cell lines, derivatives in the **10a–h** series were >2164 to >33 701 fold more selective to cancer cell lines than nonmalignant cells with **10b** showing the highest selectivity. On the other hand, compounds tested from other series **12b** and **12f** exhibited >223 and >518 fold selectivity towards the malignant breast cancer cell lines, respectively. It can be inferred from these results that the chemotypes in hand show considerable safety margin towards



Synthesis of derivatives **13-18.**

Scheme 3 Synthesis of derivatives **13–18**. Reagents and solvents: (i) $NH_2OH \cdot HCl$, pyridine; (ii) urea, anhydrous CH_3COONa, glacial acetic acid; (iii) thiourea, anhydrous CH_3COONa, glacial acetic acid; (iv) thiosemicarbazide, anhydrous CH_3COONa, glacial acetic acid; (v) appropriate phenylhydrazine, absolute ethanol; (vi) *p*-aminobenzoic acid.

nonmalignant breast cells compared to malignant breast cancer cell lines when tested under the same experimental conditions.

(c) *In vivo* cytotoxic activity. Compounds showing higher cytotoxic activity against all breast cancer cell lines, particularly MCF-7 (**10a–h** and **12a–h**) were selected to perform *in vivo* cytotoxicity and data are given in Table 3.

Results showed that all compounds were 3.89–11.23 fold more potent than the vehicle control, showing tumor volume in the range of 3.58–8.12 cm^3 as compared to the vehicle control which displayed a tumor volume of 40.21 cm^3. This is perfectly in compliance with the *in vitro* cytotoxicity data where the most active compound among the pyridinecarboxamide series bearing acetyl hydrazinyl side chain **10a** ($IC_{50} = 0.012$ nM) was also the most active *in vivo*. Interestingly, the tested compounds were also 1.12–2.56 fold more potent than the positive control drug sorafenib (tumor volume 9.1 cm^3).

It is worth mentioning that the *in vivo* experiments were tried on two doses of the tested compounds (low dose of 5 mg kg^{-1} and high dose of 10 mg kg^{-1}, every 48 h for 3 weeks) and data reported herein are for the low dose. This is because first tumor volume changes observed at the two tested doses were not significantly different. Furthermore, there was no dose-dependent toxicity in animal groups treated at the two dose levels (data not shown).

(d) Kinases inhibition assay. To investigate the mechanism of action and the kinase inhibitory profile of the target compounds, they were tested at a single dose concentration of 10 μM over a panel of 30 kinases to determine their kinase

Table 1 Cytotoxic activities of the target quinazolinones; **8a–l**, **10a–h**, **13a**, **b**, **14a**, **b**, **15a**, **b**, **16a**, **b**, **18a**, **b**, **17a–f** and *N*-(2,4-disubstituted phenyl) pyridinecarboxamides; **9a–n** and **12a–h** against breast, colon and leukemia cell lines

	IC_{50}[a] (μM)								
	Breast cancer			Colon cancer			Leukemia		
Cpd no.	MCF-7	MDA-MB-231	HS-578T	MDA-MB-435	HT-29	HCC-2998	CCRF-CEM	K-562	HL-60
8a	>100	0.0012	0.0042	>100	0.0064	0.0043	0.0575	0.0696	0.0658
8b	>100	>100	>100	>100	0.0045	0.0054	0.0484	0.087	0.0899
8c	>100	>100	>100	0.087	0.65	0.71	0.0395	0.0765	0.0640
8d	0.0009	>100	>100	0.049	0.75	0.82	0.0486	0.065	0.0273
8e	>100	0.0068	0.0065	>100	0.05	0.93	0.05679	0.054	0.0219
8f	0.00098	0.0046	0.0012	>100	0.0078	0.0065	0.0466	0.0505	0.0367
8g	0.0065	>100	>100	0.021	0.0055	0.0075	0.0579	0.0344	0.0349
8h	>100	>100	>100	0.035	0.0046	0.0070	0.0457	0.0235	0.0655
8i	>100	>100	>100	0.034	0.0057	0.0099	0.0335	0.0366	0.0894
8j	>100	>100	>100	0.023	0.0074	0.0088	0.0344	0.0478	0.0673
8k	0.0095	0.0057	0.01	>100	0.0079	0.0076	0.0455	0.0484	0.0456
8l	>100	>100	>100	0.024	0.0064	0.0034	0.0468	0.0455	0.0258
9a	0.0054	0.0047	0.0032	0.0027	0.26	0.35	0.0254	0.0268	0.3212
9b	0.0034	0.0043	0.0067	0.0016	0.15	0.24	0.0135	0.0156	0.1243
9c	0.0056	0.0078	0.0067	0.0068	0.48	0.58	0.0443	0.0467	0.0565
9d	0.0065	0.0087	0.0080	0.0045	0.65	0.36	0.0244	0.0255	0.0893
9e	0.0078	0.0090	0.0089	0.0054	0.39	0.47	0.0334	0.0366	0.0674
9f	0.0032	0.0065	0.0045	0.0039	0.57	0.46	0.0344	0.0378	0.4566
9g	0.0089	0.0053	0.0090	0.0056	0.76	0.55	0.0354	0.0755	0.0904
9h	>100	0.0065	0.0043	0.043	0.95	0.86	0.0546	0.0953	0.0566
9i	0.0065	0.0034	>100	>100	0.87	0.64	0.0455	0.0843	0.0874
9j	0.0045	0.0038	0.0046	0.034	0.06	0.97	0.0435	0.0755	0.0435
9k	>100	0.0077	45	>100	0.07	0.94	0.0565	0.0544	0.0566
9l	>100	0.0056	11	>100	0.06	0.03	0.0456	0.0654	0.0575
9m	0.0034	0.0074	0.0052	0.0043	0.05	0.96	0.0544	0.0566	0.0264
9n	>100	0.0064	67	>100	0.06	0.85	0.0478	0.043	0.0348
10a	0.000012	0.00010	0.00045	0.00028	0.0051	0.0031	0.041	0.065	0.031
10b	0.000014	0.00011	0.00057	0.00029	0.0056	0.0032	0.049	0.064	0.042
10c	0.000016	0.00012	0.00078	0.00035	0.0063	0.0033	0.058	0.076	0.053
10d	0.000018	0.00014	0.00085	0.00036	0.0072	0.0044	0.064	0.085	0.064
10e	0.000019	0.00015	0.00087	0.00044	0.0074	0.0045	0.075	0.087	0.065
10f	0.000021	0.00016	0.00095	0.00045	0.0086	0.0046	0.076	0.091	0.066
10g	0.000022	0.00017	0.00097	0.00046	0.0095	0.0048	0.087	0.096	0.067
10h	0.000023	0.00022	0.00099	0.00048	0.0099	0.0049	0.098	0.099	0.068
12a	0.00012	0.0035	0.0044	0.0035	0.025	0.035	0.0013	0.0023	0.0019
12b	0.00024	0.0043	0.0055	0.0046	0.035	0.034	0.0022	0.0034	0.0028
12c	0.00035	0.0056	0.0046	0.0047	0.026	0.057	0.0035	0.0046	0.0037
12d	0.00046	0.0078	0.0055	0.0058	0.017	0.065	0.0024	0.0035	0.0046
12e	0.00054	0.0098	0.0074	0.0067	0.036	0.034	0.0043	0.0027	0.0059
12f	0.00064	0.0054	0.0083	0.0056	0.045	0.036	0.0056	0.0038	0.0048
12g	0.00076	0.0026	0.0075	0.0045	0.054	0.055	0.0065	0.0057	0.0034
12h	0.00078	0.0045	0.0066	0.0036	0.043	0.046	0.0057	0.0066	0.0023
13a	0.0023	0.0032	0.0047	0.0078	0.034	0.054	0.0078	0.0075	0.0072
13b	0.0043	0.0045	0.0058	0.0063	0.075	0.023	0.0069	0.0084	0.0064
14a	0.0054	0.0076	0.0069	0.0052	0.086	0.014	0.0058	0.0093	0.0096
14b	0.0067	0.0053	0.0078	0.0083	0.075	0.023	0.0046	0.0084	0.0087
15a	0.0065	0.0045	0.0087	0.0091	0.098	0.054	0.0065	0.0095	0.0096
15b	0.0043	0.0067	0.0075	0.0082	0.087	0.067	0.0054	0.0076	0.0085
16a	0.0023	0.0089	0.0064	0.0093	0.058	0.056	0.0043	0.0047	0.0053
16b	0.0045	0.0087	0.0054	0.0087	0.036	0.043	0.0054	0.0056	0.0044
17a	0.0045	0.0033	0.0054	0.0078	0.054	0.067	0.0065	0.0035	0.0065
17b	0.0034	0.0044	0.004	0.0089	0.085	0.067	0.0074	0.0043	0.0076
17c	0.0056	0.0053	0.003	0.0036	0.076	0.089	0.0085	0.0054	0.0057
17d	0.0078	0.0032	0.004	0.0045	0.057	0.087	0.0095	0.0065	0.0038
17e	0.0075	0.0045	0.005	0.0054	0.068	0.053	0.0086	0.0076	0.0049
17f	0.0045	0.0054	0.004	0.0065	0.076	0.045	0.0077	0.0047	0.0059
18a	0.0034	0.0065	0.005	0.0056	0.024	0.045	0.0066	0.0035	0.0048
18b	0.0034	0.0074	0.004	0.0045	0.045	0.034	0.0055	0.0023	0.0038

[a] The average IC_{50} values were calculated from three independent tests.

Table 2 *In vitro* cytotoxicity of derivatives **10a–h**, **12b** and **12f** against MCF-10A

Cpd no.	IC_{50} (μM)	Selectivity[b]	Cpd no.	IC_{50} (μM)	Selectivity
10a	12.09	>26 866	**12a**	NT[a]	NT
10b	19.21	>33 701	**12b**	1.23	>223
10c	2.10	>2692	**12c**	NT	NT
10d	3.20	>3764	**12d**	NT	NT
10e	1.98	>2275	**12e**	NT	NT
10f	2.30	>2421	**12f**	4.30	>518
10g	2.10	>2164	**12g**	NT	NT
10h	3.30	>3333	**12h**	NT	NT

[a] NT: not tested. [b] Selectivity in fold relative to the lowest observed potency among tested breast cancer cell lines.

activity percent inhibition. The synthesized compounds exerted multiple inhibitions over the tested oncogenic protein kinases at the tested concentration. The synthesized compounds completely inhibited the enzymatic activity of ABL, ALK and c-RAF kinases. On the other hand, the inhibitions were of low percent in the other tested 27 kinases.

For those kinases that were 100% inhibited at the initial high dose, *viz.* ABL, ALK and c-RAF, IC_{50} values of the test compounds were determined. Kinase activity was assessed using HotSpot^{SM} Technology, a miniaturized radio-isotope based filter binding assay.[29]

Abelson kinase (ABL) is a fusion tyrosine kinase playing a role in 90% of chronic myeloid leukemia (CML) cases.[30] Anaplastic Lymphoma Kinase (ALK) is a member of the insulin receptor tyrosine kinase family.[31] It is constitutively active and plays an oncogenic role in 70–80% of all anaplastic large cell lymphomas.[32] On the other hand, over-expression of ALK was observed in glioblastoma[33] and neuroblastoma.[34] Moreover, dysregulated signaling through RAF kinase isoforms has been detected in ~30% of human cancers where wild type c-RAF is hyperactivated in a wide range of human solid tumors.[35]

The kinase inhibitory activities (nM) of the targeted quinazolinones; **8a–l**, **10a–h**, **(13–16, 18)a,b** and **17a–f** and *N*-(2,4-disubstituted phenyl)pyridinecarboxamides; **9a–n** and **12a–h** against ABL kinase (100% inhibited) are listed in Table 4.

Interestingly, the target quinazolinones; **8a–l**, **10a–h** and **13–18** as well as the substituted *N*-(2,4-disubstituted phenyl)pyridinecarboxamides; **9a–n** and **12a–h** exhibited a wide range of ABL, ALK and c-RAF inhibitory activity with IC_{50} ranges 0.011–76 nM and 0.24–55 nM, 0.05–323 nM and 0.09–93 nM, 0.042–66 nM and 0.1–79 nM, respectively. The quinazolinyl-3-urea/thiourea derivatives **8a–l** and the pyridinecarboxamides with benzylidene-carrying side chain **9a–n** displayed moderate to very weak inhibitory activity against ABL (IC_{50} = 17–76 nM), ALK (IC_{50} = 19–323 nM) and c-RAF kinases (IC_{50} = 24 nM-inactive). The pyridinecarboxamides bearing acetyl hydrazinyl-carrying side chain **12a–h** and the 3-substituted quinazolin-4(3*H*)-one **13–18** had higher potency against ABL, ALK and c-RAF kinases (IC_{50} = 0.24–0.79 nM, 0.09–0.89 nM and 0.1–0.97, respectively). Additionally, the 3-substituted benzylidene quinazolinones **10a–h** showed exceptional ABL, ALK and c-RAF inhibitory potency (IC_{50} = 0.011–0.037 nM, 0.05–0.074 nM, 0.042–0.057 nM, respectively).

Concerning the quinazolinone derivatives **8a–l**: generally, replacement of the pyridin-3-yl moiety (**8a–f**) by pyridin-4-yl moiety (**8g–l**) caused a slight increase in ABL inhibitory activity and it did not have a significant effect on ALK inhibitory activity. On the other hand, it nearly abolished c-RAF activity. Surprisingly, side chain shortening of the phenylureido

Table 3 MCF-7 mouse xenograft model of breast cancer for compounds **10a–h** and **12a–h**

	Tumor growth V_t/V_o for compounds at time[a] (days)										
Compound no.	0	2	4	6	8	10	12	14	16	18	20
Vehicle control	1.0 ± 0.01[b]	1.3 ± 0.07	1.8 ± 0.07	4.7 ± 0.2	9.8 ± 0.5	12.6 ± 1.4	24.7 ± 2.7	27.6 ± 1.2	29.0 ± 1.7	38.9 ± 1.2	40.2 ± 1.8
Sorafenib	1.0 ± 0.04	1.1 ± 0.06	1.2 ± 0.06	1.5 ± 0.02	2.1 ± 0.2	2.2 ± 0.1	4.5 ± 0.2	5.5 ± 0.3	6.89 ± 0.5	8.8 ± 0.7	9.1 ± 0.3
10a	1.0 ± 0.05	1.0 ± 0.08	1.0 ± 0.07	1.1 ± 0.01	1.1 ± 0.1	1.3 ± 0.2	2.0 ± 0.3	2.6 ± 0.4	3.10 ± 0.4	3.3 ± 0.3	3.5 ± 0.2
10b	1.0 ± 0.03	1.0 ± 0.09	1.0 ± 0.08	1.1 ± 0.02	1.3 ± 0.1	1.4 ± 0.1	2.1 ± 0.4	2.7 ± 0.5	3.16 ± 0.5	3.3 ± 0.2	3.8 ± 0.2
10c	1.0 ± 0.05	1.0 ± 0.08	1.0 ± 0.07	1.1 ± 0.03	1.4 ± 0.1	1.4 ± 0.1	2.2 ± 0.3	2.8 ± 0.3	3.32 ± 0.6	4.1 ± 0.3	4.5 ± 0.1
10d	1.0 ± 0.06	1.0 ± 0.07	1.0 ± 0.08	1.1 ± 0.04	1.5 ± 0.2	1.4 ± 0.2	2.3 ± 0.2	2.9 ± 0.2	3.52 ± 0.4	4.2 ± 0.4	4.8 ± 0.4
10e	1.0 ± 0.07	1.0 ± 0.06	1.0 ± 0.09	1.1 ± 0.05	1.6 ± 0.2	1.6 ± 0.1	2.3 ± 0.3	3.0 ± 0.3	3.74 ± 0.3	4.5 ± 0.3	4.9 ± 0.5
10f	1.0 ± 0.06	1.0 ± 0.07	1.0 ± 0.08	1.2 ± 0.04	1.6 ± 0.1	1.7 ± 0.1	2.5 ± 0.2	3.1 ± 0.4	3.90 ± 0.4	4.6 ± 0.5	5.0 ± 0.4
10g	1.0 ± 0.07	1.0 ± 0.06	1.0 ± 0.07	1.2 ± 0.06	1.7 ± 0.2	1.8 ± 0.1	2.6 ± 0.3	3.2 ± 0.5	4.04 ± 0.2	4.7 ± 0.6	5.0 ± 0.5
10h	1.0 ± 0.06	1.0 ± 0.07	1.1 ± 0.08	1.2 ± 0.05	1.8 ± 0.1	1.9 ± 0.1	2.7 ± 0.2	3.5 ± 0.3	4.19 ± 0.5	4.8 ± 0.7	5.1 ± 0.3
12a	1.0 ± 0.07	1.0 ± 0.06	1.1 ± 0.07	1.2 ± 0.06	1.5 ± 0.1	1.7 ± 0.1	2.0 ± 0.3	2.5 ± 0.2	2.98 ± 0.1	3.5 ± 0.8	4.3 ± 0.2
12b	1.0 ± 0.06	1.0 ± 0.07	1.1 ± 0.06	1.2 ± 0.07	1.6 ± 0.1	1.8 ± 0.2	2.1 ± 0.2	2.6 ± 0.2	3.10 ± 0.2	4.8 ± 0.8	5.4 ± 0.6
12c	1.0 ± 0.05	1.0 ± 0.06	1.1 ± 0.06	1.2 ± 0.08	1.7 ± 0.2	1.9 ± 0.2	2.2 ± 0.1	2.7 ± 0.3	3.34 ± 0.4	4.3 ± 0.7	5.8 ± 0.5
12d	1.0 ± 0.06	1.1 ± 0.05	1.1 ± 0.04	1.3 ± 0.07	1.8 ± 0.1	1.9 ± 0.2	2.3 ± 0.2	2.8 ± 0.1	3.56 ± 0.3	4.6 ± 0.4	6.3 ± 0.6
12e	1.0 ± 0.05	1.1 ± 0.04	1.2 ± 0.03	1.3 ± 0.03	1.8 ± 0.1	2.0 ± 0.3	2.5 ± 0.1	3.2 ± 0.2	4.11 ± 0.5	5.3 ± 0.5	6.5 ± 0.7
12f	1.0 ± 0.04	1.1 ± 0.06	1.2 ± 0.04	1.4 ± 0.04	1.9 ± 0.2	2.1 ± 0.2	3.0 ± 0.4	3.6 ± 0.3	4.48 ± 0.4	5.6 ± 0.6	6.8 ± 0.8
12g	1.0 ± 0.03	1.1 ± 0.07	1.2 ± 0.05	1.4 ± 0.05	1.9 ± 0.1	2.1 ± 0.3	3.2 ± 0.3	4.2 ± 0.4	5.23 ± 0.5	5.9 ± 0.7	7.1 ± 0.7
12h	1.0 ± 0.04	1.1 ± 0.06	1.3 ± 0.06	1.5 ± 0.06	2.0 ± 0.2	2.2 ± 0.2	3.5 ± 0.2	4.5 ± 0.5	5.67 ± 0.7	6.1 ± 0.8	8.1 ± 0.9

[a] Tested compounds and the control drug sorafenib were administered by intraperitoneal (i.p.) injection at a dose of 5 mg kg^{-1}, every 48 h for 3 weeks. [b] Each data point is the mean ± SEM of five animals.

Table 4 Kinase inhibitory activity

	IC_{50} (nM)				IC_{50} (nM)		
Compound number	ABL	ALK	c-RAF	Compound number	ABL	ALK	c-Raf
8a	54	64	64	**10d**	0.023	0.065	0.046
8b	43	72	53	**10e**	0.035	0.067	0.057
8c	66	21	62	**10f**	0.037	0.074	0.057
8d	76	35	43	**10g**	0.036	0.076	0.058
8e	68	76	34	**10h**	0.037	0.078	0.059
8f	43	56	55	**12a**	0.26	0.35	0.55
8g	44	37	Inactive	**12b**	0.35	0.43	0.44
8h	33	25	Inactive	**12c**	0.44	0.65	0.35
8i	26	323	Inactive	**12d**	0.33	0.78	0.26
8j	32	34	Inactive	**12e**	0.24	0.76	0.19
8k	47	47	66	**12f**	0.44	0.45	0.18
8l	55	50	Inactive	**12g**	0.35	0.24	0.10
9a	17	84	36	**12h**	0.26	0.35	0.17
9b	24	93	43	**13a**	0.57	0.35	0.14
9c	35	65	24	**13b**	0.46	0.43	0.13
9d	39	46	56	**14a**	0.65	0.54	0.45
9e	48	54	35	**14b**	0.74	0.32	0.36
9f	26	75	45	**15a**	0.65	0.67	0.48
9g	20	37	67	**15b**	0.54	0.89	0.56
9h	36	59	40	**16a**	0.45	0.09	0.44
9i	18	28	58	**16b**	0.56	0.76	0.55
9j	21	30	79	**17a**	0.65	0.45	0.76
9k	46	58	36	**17b**	0.76	0.34	0.84
9l	39	19	47	**17c**	0.67	0.56	0.95
9m	40	20	68	**17d**	0.79	0.54	0.86
9n	55	67	25	**17e**	0.60	0.35	0.97
10a	0.011	0.050	0.042	**17f**	0.59	0.34	0.85
10b	0.021	0.056	0.044	**18a**	0.46	0.56	0.76
10c	0.022	0.068	0.045	**18b**	0.37	0.78	0.65
Imatinib	0.58	—	0.99	Bosutinib	0.03	—	—
Staurosporine	—	0.0022	—	Sorafenib	—	—	6

derivatives **8d**, **j** to phenyl amino ones **17a**, **d** enhanced the inhibitory potency by 50–100 fold. Looking at the effect of removing the nitrogen spacer between the quinazolinone core and the side chain linked to the quinazolinyl-N3, it was found that this structural modification could be beneficial for the ABL, ALK and c-RAF kinases inhibitory activity (IC_{50} = 0.37–0.79, 0.09–0.89 and 0.13–0.97 nM, respectively) in the quinazolinones **13**, **14**, **15**, **16**, **18a**, **b**. With respect to the pyridinecarboxamides with benzylidene-carrying side chain **9a–n**, it was observed that this series displayed slightly higher activity than the quinazolinones **8a–l**.

Rigidification of the Schiff's bases **9a–n** through cyclodehydration afforded the most potent 3-substituted quinazolinones in this work **10a–h** which showed exceptional enzyme inhibitory potency against ABL, ALK and c-RAF (IC_{50} = 0.011–0.037 nM, 0.05–0.074 nM, 0.042–0.057 nM, respectively) which is consistent with their antitumor activity. Exploring the effect of different substituents on the benzylidene moiety, revealed that decreasing the size and increasing the electronegativity of the halogenated substituent resulted in improving the potency in case of 3-pyridyl analogues. Therefore, the fluoro derivative **10a** displayed higher activity than the chloro analogue **10b** which in turn exhibited slightly higher activity than the bromo derivative **10c** (IC_{50} = 0.011, 0.021, 0.022 nM, respectively in case of ABL), (IC_{50} = 0.050, 0.056, 0.068 nM, respectively in case of ALK), (IC_{50} = 0.042, 0.044, 0.045 nM, respectively in case of c-RAF). Similar results were observed for the 4-pyridyl derivatives. It is worth to mention that the fluoro-3-pyridyl derivative **10a** exhibited the most promising kinase inhibitory activity in this study.

Finally, as evidenced from the experimental data showed, the most active kinase activity inhibiting series in this study **10a–h** were equi- to many folds more potent than the reference kinase inhibitors against their target kinases *viz.* imatinib (ABL and c-RAF), bosutinib (ABL), sorafenib (c-RAF) and staurosporine (ALK).

(2) Molecular modelling

The docking study was initiated to predict the interaction of the newly synthesized compounds against the kinase ABL towards which they showed the highest enzymatic activity inhibition. The two PDB files 2HYY and 3UE4 representing inactive DFG-out conformations of ABL-co-crystallized with imatinib and bosutinib, respectively.[36,37] It is reported that the two conformations are distinct in the sense that the in the latter crystal structure of ABL with its inhibitor bosutinib, the activation loop adopts a conformation like that observed in active kinases,

except for the conformation of the DFG motif itself. This is in contrast with the commonly known conformational orientation of the activation loop seen in case of the type II inhibitor imatinib (PDB entry 2HYY) in which the P-loop folds over the ATP binding site, and the activation-loop blocks the substrate binding site with a shift of the DFG motif nearer to the front of the active site.

Docking of the most active compounds **10a–h** and **12a–h** was performed on both crystal–inhibitor complexes to predict whether these derivatives elicit their activity by binding to the active pocket with a behavior compared to that of imitanib or bosutinib. Results showed that while the type II inhibitor imatinib displayed a network of hydrogen bonds to anchor itself tightly with the ABL binding cleft residues, all attempts to dock the new chemotypes in the active site of ABL DFG-out conformation were unsuccessful due to multiple steric clashes with the active site residues (data not shown). On the other hand, docking calculations based on the ABL kinase domain conformation induced by binding of bosutinib to ABL (PDB entry 3UE4, quinazoline N and Met318 amide NH hydrogen bond of 1.9 Å, $S = -18.42$ kcal mol^{-1}) gave rise to complexes with comparable binding modes and energetics to that of the co-crystallized ligand bosutinib. Results displayed herein are those obtained for attempted docking of the most active compound **10a** (IC_{50} = 0.011 nM) where Fig. 2 shows the respective 2D and 3D interaction contacts of the top scoring and best fitting pose obtained through this molecular modeling study. The highest ABL inhibitory activity of the Schiff's base derivative **10a** might be attributed to high ABL binding, energy score ($S = -21.22$ kcal mol^{-1}) where the 3D interactions (Fig. 2: bottom panel) showed that the pyridyl N atom formed a hydrogen bond with the amide NH of the gatekeeper Met318 (2.4 Å). It is noteworthy that this interaction is considered the key contributor to the overall strength of drug–ABL kinase complexes.[38–40] Additionally, the carbonyl O of the quinazolin-4-one ring interacted with the amidic NH of the Asn322 through a hydrogen (2.1 Å). These results along with the other obtained but not displayed ones strongly suggest that our compounds probably act as type II kinase inhibitors by binding to the DFG out conformation of the catalytic domain of ABL.

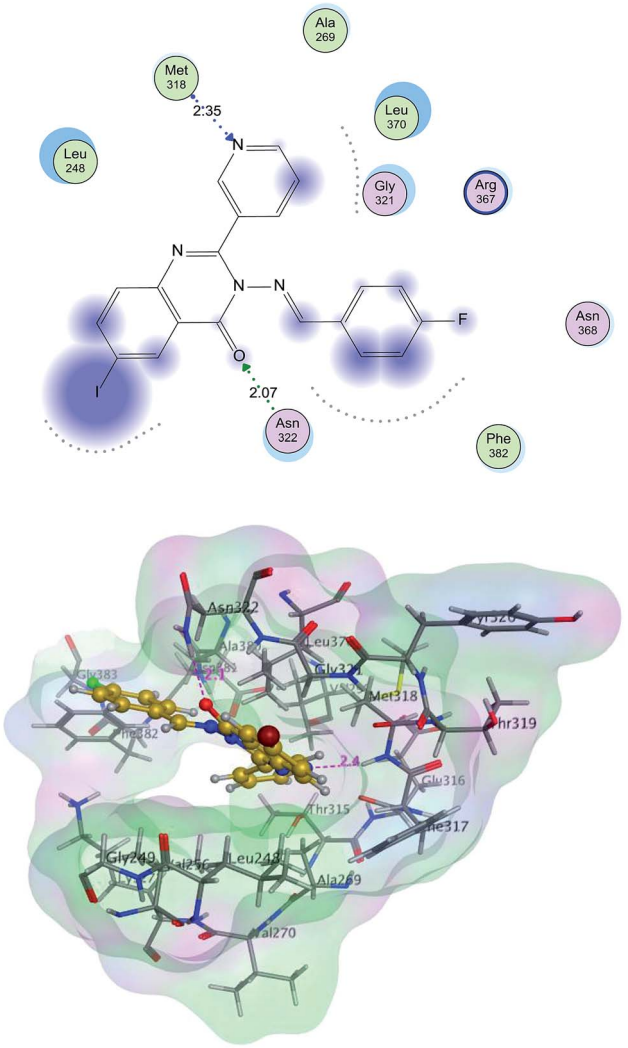


Fig. 2 Top panel: 2D interactions; bottom panel: 3D interactions of **10a** and ABL (PDB entry 3UE4).

Conclusion

The present study reports the design and synthesis of five novel series of 6-iodo-2-(pyridin-3/4-yl)-3-substituted quinazolin-4(3*H*)-one and acyclic analogues as multi-kinase inhibitors. The design was based on structural analogy between the new compounds, reported quinazoline derivatives and acyclic analogues. Synthesis of the target compounds was carried out employing techniques reported in the literature with some modifications when necessary. This led to the synthesis of 8 new intermediates and 58 new final compounds. The structures of the synthesized compounds were confirmed by various spectral analysis techniques as well as the elemental analyses.

The target compounds were evaluated for their antitumor activity against a panel of nine cancer cell lines. This panel involved breast cancers (MCF-7, MDAMB-231, MDAMB-435 and HS-578T), colon cancers (HT-29 and HCC-2998) and leukemia cell lines (CCRF-CEM, K-562 and HL-60). The biological results revealed that target quinazolinones **8a–l**, **10a–h**, **13a**, **b**, **14a**, **b**, **15a**, **b**, **16a**, **b**, **17a–f** and **18a**, **b**, and *N*-(2,4-disubstituted phenyl) pyridinecarboxamides **9a–n** and **12a–h** exhibited potent antitumor activity. Aiming to investigate their mechanism of action, the synthesized were tested over a panel of 30 kinases; ABL, ALK and c-RAF were the most responsive kinases. Molecular docking was carried out to predict the binding mode of these chemotypes with the active site amino acids of the ABL, being the most responsive kinase to the inhibitory effect of the tested compounds. Molecular modeling results showed that these derivatives recognize the active pocket of the target enzyme selectively to a more relaxed conformation of the ABL active site as induced by the known inhibitor bosutinib. In conclusion, the synthesized compounds, especially **10a**, can be viewed as promising leads for further investigation and optimization as multi-kinase inhibitors.

Experimental section

Melting points were determined on Electrothermal Stuart SMP_3 digital melting point apparatus and were uncorrected. Elemental microanalyses were performed at the Regional Center for Mycology and Biotechnology, Al-Azhar University and at the Microanalytical Centre, Faculty of Science, Cairo University. Infrared spectra were determined (KBr) using Shimadzu Infrared spectrometer (IR-435) and FT-IR 1650 (Perkin Elmer), Faculty of Pharmacy, Cairo University. ^{1}H-NMR spectra were performed in $CDCl_3$ or DMSO-d_6 using Joel, 300 MHz NMR spectrometer, Faculty of Science, Cairo University, and Bruker, 400 MHz NMR spectrometer, Microanalytical unit, Faculty of Pharmacy, Cairo University. ^{13}C-NMR spectra were recorded using Bruker, 100 MHz NMR spectrometer, Microanalytical unit, Faculty of Pharmacy, Cairo University. Mass spectra were carried out using Finnigan SSQ 7000 Gas chromatograph-Mass, at the Microanalytical Centre, Faculty of Science, Cairo University. Thin layer chromatography was carried out on silica gel TLC plates with fluorescence indicator (F_{254}). 5-Iodoanthranilic acid (**3**),[26] nicotinoyl chloride (**4a**),[27] isonicotinoyl chloride (**4b**)[28] were prepared according to the reported method.

(1) Chemistry

5-Iodo-2-[(pyridin-3-ylcarbonyl)amino]benzoic acid (5a). A solution of 5-iodoanthranilic acid **3** (2.63 g, 10 mmol) in dry dimethylformamide (20 mL) was added to the freshly prepared nicotinoyl chloride hydrochloride (2.13 g, 12 mmol) and the mixture was heated at 80 °C for 2 h, and then allowed to cool. The reaction mixture was poured onto ice-water. The solid obtained was filtered, washed several times with water and dried to give 3.2 g (71%) which was recrystallized from acetic acid. Mp: 314–316 °C. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3435–3414 (br.) (OH), 3338 (NH), 1676, 1664 (2C=O), 1608 (C=N), 1519 (C=C), 530 (C–I). ^{1}H-NMR 300 MHz (DMSO-d_6, D_2O ppm): δ 7.66 (dd, 1H, J = 4.80, 7.80 Hz, pyridyl-H), 8.00 (dd, 1H, J = 2.10, 8.70 Hz, phenyl-H), 8.29 (t, 1H, J = 2.40 Hz, pyridyl-H), 8.32 (dd, 1H, J = 1.80, 6.30 Hz, phenyl-H), 8.40 (d, 1H, J = 8.70 Hz, phenyl-H), 8.83 (dd, 1H, J = 1.50, 4.80 Hz, pyridyl-H), 9.11 (d, 1H, J = 1.80 Hz, pyridyl-H), 9.30 (s, 1H, NH exchanged by D_2O), 12.02 (s, 1H, OH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 368 (M^+, 44.38%), 106 (100%). Anal. calcd for $C_{13}H_9IN_2O_3$ (368.13): C, 42.41; H, 2.46; N, 7.61. Found: C, 42.64; H, 2.45; N, 7.70.

5-Iodo-2-[(pyridin-4-ylcarbonyl)amino]benzoic acid (5b). Compound **5b** was prepared and purified by adopting the same procedure used for **5a** using isonicotinoyl chloride. Mp: 290–292 °C. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3232 (OH), 3145 (NH), 1683, 1653 (2C=O), 1595 (C=N), 1573 (C=C), 526 (C–I). ^{1}H-NMR 300 MHz (DMSO-d_6, D_2O ppm): δ 7.96 (d, 2H, J = 4.80 Hz, pyridyl-H), 7.99 (dd, 1H, J = 6.00, 8.10 Hz, phenyl-H), 8.29 (d, 1H, J = 1.80 Hz, phenyl-H), 8.38 (dd, 1H, J = 3.00, 8.70 Hz, phenyl-H), 8.92 (d, 2H, J = 4.80 Hz, pyridyl-H), 10.82 (s, 1H, NH exchanged by D_2O), 12.11 (s, 1H, OH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 368 (M^+, 34.7%), 106 (100%). Anal. calcd for $C_{13}H_9IN_2O_3$ (368.13): C, 42.41; H, 2.46; N, 7.61. Found: C, 42.57; H, 2.48; N, 7.73.

6-Iodo-2-(pyridin-3-yl)-4*H*-benzo[*d*][1,3]oxazin-4-one (6a)

Method 1. A mixture of the amidated anthranilic acid **5a** (1.84 g, 5 mmol) in acetic anhydride (15 mL) was heated under reflux for 5 h. Then the reaction mixture was poured onto ice-water. The obtained beige solid product was collected by filtration, dried to give 1.4 g (84%) and then recrystallized from methanol. Mp: 167–169 °C. Yield: 84%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3049 (CH aromatic), 1753 (C=O), 1626 (C=N), 1585 (C=C), 530 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, ppm): δ 7.53 (d, 1H, J = 8.44 Hz, phenyl-H), 7.64 (dd, 1H, J = 4.84, 8.04 Hz, pyridyl-H), 8.27 (dd, 1H, J = 2.00, 8.44 Hz, phenyl-H), 8.42 (d, 1H, J = 1.96 Hz, phenyl-H), 8.49 (t, 1H, J = 8.04 Hz, pyridyl-H), 8.83 (dd, 1H, J = 1.60, 4.80 Hz, pyridyl-H), 9.30 (d, 1H, J = 2.20 Hz, pyridyl-H). Mass (*m*/*z*, rel. abundance): 350 (M^+, 100%). Anal. calcd for $C_{13}H_7IN_2O_2$ (350.11): C, 44.60; H, 2.02; N, 8.00. Found: C, 44.92; H, 1.85; N, 8.08.

Method 2. A solution of 5-iodoanthranilic acid (2.63 g, 10 mmol) in dry pyridine (30 mL) was added to the freshly prepared isonicotinoyl chloride hydrochloride (2.13 g, 12 mmol) and the mixture was stirred at 5 °C for 2 h, and then allowed to warm to room temperature. The reaction mixture was poured onto ice-water, the solid obtained was filtered, washed with diluted acetic acid, dried and recrystallized from methanol. Mp: 167–169 °C. Yield: 65%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3049 (CH aromatic), 1753 (C=O), 1626 (C=N), 1585 (C=C), 530 (C–I).

6-Iodo-2-(pyridin-4-yl)-4*H*-benzo[*d*][1,3]oxazin-4-one (6b)

Method 1. This compound was prepared according to method 1 used for preparation of **6a** but using **5b** as a starting material and was also recrystallized from methanol.

Mp: 185–187 °C. Yield: 75%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3057 (CH aromatic), 1764 (C=O), 1616 (C=N), 1556 (C=C), 534 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, ppm): δ 7.50 (d, 1H, J = 8.40 Hz, phenyl-H), 7.99 (d, 2H, J = 6.04 Hz, pyridyl-H), 8.23 (dd, 1H, J = 2.00, 8.40 Hz, phenyl-H), 8.37 (d, 1H, J = 1.88 Hz, phenyl-H), 8.78 (d, 2H, J = 6.04 Hz, pyridyl-H). Mass (*m*/*z*, rel. abundance): 350 (M^+, 100%). Anal. calcd for $C_{13}H_7IN_2O_2$ (350.11): C, 44.60; H, 2.02; N, 8.00. Found: C, 44.54; H, 1.81; N, 8.09.

Method 2. Method 2 used for preparation of **6a** was also used for synthesis of **6b** using **5b** as a starting material.

Mp: 185–187 °C. Yield: 55%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3057 (CH aromatic), 1764 (C=O), 1616 (C=N), 1556 (C=C), 534 (C–I).

***N*-[2-(Hydrazinylcarbonyl)-4-iodophenyl]pyridine-3-carboxamide (7a).** A mixture of benzoxazinone **6a** (1.74 g, 5 mmol) and hydrazine hydrate 99% (15 mmol, 0.8 mL) in absolute ethanol (20 mL) was refluxed for 4 h, left to cool then filtered. The separated white solid was collected, washed with ethanol and recrystallized from ethyl acetate. Mp: 221–223 °C. Yield: 75%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3309 (2NH), 3213, 3151 (NH_2), 3061 (CH aromatic), 1697 (2C=O), 1631 (C=N), 1516 (C=C), 530 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 4.70 (s, 2H, NH_2 exchanged by D_2O), 7.62 (dd, 1H, J = 4.88, 7.96 Hz, pyridyl-H), 7.90 (dd, 1H, J = 1.32, 8.80 Hz, phenyl-H), 8.11 (s, 1H, phenyl-H), 8.26 (t, 1H, J = 8.04 Hz, pyridyl-H), 8.39 (d, 1H, J = 8.8 Hz, phenyl-H), 8.80 (dd, 1H, J = 1.52, 4.80 Hz, pyridyl-H), 9.10 (d, 1H, J = 1.84 Hz, pyridyl-H), 10.29 (s, 1H, NH exchanged by D_2O), 12.49 (s, 1H, NH exchanged by D_2O). Mass

(*m*/*z*, rel. abundance): 382 (M^+, 1.63%), 216 (100%). Anal. calcd for $C_{13}H_{11}IN_4O_2$ (382.16): C, 40.86; H, 2.90; N, 14.66. Found: C, 40.98; H, 2.96; N, 14.89.

***N*-[2-(Hydrazinylcarbonyl)-4-iodophenyl]pyridine-4-carboxamide (7b).** Compound **7b** was prepared and purified by using the same procedure adopted for the preparation of **7a** but using **6b** as a starting material. Mp: 238–240 °C. Yield: 75%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3250 (2NH), 3165, 3130 (NH_2), 3074 (CH aromatic), 1681 (C=O), 1638 (C=N), 1517–1434 (C=C), 530 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 4.72 (s, 2H, NH_2 exchanged by D_2O), 7.82 (d, 2H, J = 5.96 Hz, pyridyl-H), 7.91 (dd, 1H, J = 1.56, 8.76 Hz, phenyl-H), 8.13 (s, 1H, phenyl-H), 8.40 (d, 1H, J = 8.76 Hz, phenyl-H), 8.85 (d, 2H, J = 5.96 Hz, pyridyl-H), 10.32 (s, 1H, NH exchanged by D_2O), 12.60 (s, 1H, NH exchanged by D_2O). Anal. calcd for $C_{13}H_{11}IN_4O_2$ (382.16): C, 40.86; H, 2.90; N, 14.66. Found: C, 40.98; H, 2.93; N, 14.82.

1-(6-Iodo-4-oxo-2-(pyridin-3-yl) quinazolin-3(4*H*)-yl)-3-substituted urea/thiourea derivatives (8a–f)

General method. A mixture of the hydrazido derivative **7a** (0.72 g, 2 mmol) and the appropriate alkyl or aryl isocyanate (2.2 mmol) or isothiocyanate (3 mmol) in dioxane (20 mL) was refluxed for 6–10 h. Then allowed to cool. The precipitated solid product was collected by filtration, washed with aqueous ethanol then dried and recrystallized from absolute ethanol to give **8a–f**.

*1-Ethyl-3-(6-iodo-4-oxo-2-(pyridin-3-yl)quinazolin-3(4H)-yl)thiourea (**8a**).* Mp: 210–211 °C. Yield: 85%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3300, 3251 (2NH), 3215 (CH aromatic), 2972, 2926 (CH_2, CH_3), 1681 (C=O), 1654 (C=N), 1508 (C=C), 1215 (C=S), 530 (C–I). ^{1}H-NMR 300 MHz (DMSO-d_6, D_2O, ppm): δ 1.02 (t, 3H, J = 6.90 Hz, CH_3CH_2), 3.45 (q, 2H, J = 6.90 Hz, CH_3CH_2), 7.62 (dd, 1H, J = 4.80, 8.10 Hz, pyridyl-H), 7.98 (d, J = 2.10 Hz, quinazolinyl), 8.13–8.24 (m, 3H, quinazolinyl-H, pyridyl-H), 8.81 (dd, 1H, J = 1.8, 4.80 Hz, 1H, pyridyl-H), 9.06 (d, 1H, J = 2.40 Hz, pyridyl-H), 10.7 (s, 1H, NH exchanged by D_2O), 11.68 (s, 1H, NH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 451 (M^+, 53.61%), 171 (100%). Anal. calcd for $C_{16}H_{14}IN_5OS$ (451.28): C, 42.58; H, 3.13; N, 15.52. Found: C, 42.33; H, 3.45; N, 15.26.

*1-Cyclohexyl-3-(6-iodo-4-oxo-2-(pyridin-3-yl)quinazolin-3(4H)-yl)urea (**8b**).* Mp: 235–237 °C. Yield: 80%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3327, 3296 (2NH), 2935 (CH_2 aliphatic), 1687, 1660 (2C=O), 1647 (C=N), 1506–1440 (C=C), 530 (C–I). ^{1}H-NMR 300 MHz (DMSO-d_6, D_2O, ppm): δ 1.07–1.59 (m, 10H, cyclohexyl-5CH_2), 3.35 (m, 1H, cyclohexyl-CH), 7.58 (dd, 1H, J = 4.8 Hz, 7.8 Hz, quinazolinyl-H), 7.86 (s, 1H, pyridyl-H), 7.96 (d, 1H, J = 2.1 Hz, quinazolinyl-H), 8.10 (s, 1H, quinazolinyl-H), 8.19–8.22 (m, 1H, pyridyl-H), 8.80 (dd, 1H, J = 1.8, 5.1 Hz, pyridyl-H), 9.05 (s, 1H, pyridyl-H), 10.44 (s, 1H, NH exchanged by D_2O), 11.78 (s, 1H, NH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 489 (M^+, 5.6%), 106.05 (100%). Anal. calcd for $C_{20}H_{20}IN_5O_2$ (489.31): C, 49.09; H, 4.12; N, 14.31. Found: C, 49.16; H, 4.18; N, 14.58.

*1-Cyclohexyl-3-(6-iodo-4-oxo-2-(pyridin-3-yl)quinazolin-3(4H)-yl)thiourea (**8c**).* Mp: 232–234 °C. Yield: 79%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3404, 3309 (2NH), 2924 (CH_2 aliphatic), 1676 (C=O), 1649 (C=N), 1516–1448 (C=C), 1201 (C=S), 530 (C–I). ^{1}H-NMR 300 MHz (DMSO-d_6, D_2O, ppm): δ 1.01–1.75 (m, 10H, cyclohexyl-5CH_2), 4.06 (m, 1H, cyclohexyl-CH), 7.52 (t, 1H, J = 6.3 Hz, pyridyl-H), 7.59 (dd, 1H, J = 4.8, 8.1 Hz, quinazolinyl-H), 7.95 (s, 1H, quinazolinyl-H), 8.14 (d, 1H, J = 6.9 Hz, pyridyl-H), 8.21 (d, 1H, J = 8.4 Hz, quinazolinyl-H), 8.79 (d, 1H, J = 3.3 Hz, pyridyl-H), 9.04 (s, 1H, pyridyl-H), 10.59 (s, 1H, NH exchanged by D_2O), 11.5 (s, 1H, NH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 505.3 (M^+, 3.49%), 106.05 (100%). Anal. calcd for $C_{20}H_{20}IN_5OS$ (505.38): C, 47.53; H, 3.99; N, 13.86. Found: C, 47.49; H, 4.17; N, 14.11.

*1-(6-Iodo-4-oxo-2-(pyridin-3-yl)quinazolin-3(4H)-yl)-3-phenylurea (**8d**).* Mp: 230–232 °C. Yield: 89% I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3296 (2NH), 3101 (CH aromatic), 1683, 1668 (2C=O), 1639 (C=N), 1506–1446 (C=C), 530 (C–I). ^{1}H-NMR 300 MHz (DMSO-d_6, D_2O, ppm): δ 6.99 (t, 1H, J = 7.5 Hz, phenyl-H), 7.25 (t, 2H, J = 7.5 Hz, phenyl-H), 7.45 (d, 2H, J = 8.4 Hz, phenyl-H), 7.57 (dd, 1H, J = 5.1 Hz, 7.8 Hz, pyridyl-H), 7.97 (dd, 1H, J = 2.1 Hz, 9.00 Hz, quinazolinyl-H), 8.18–8.24 (m, 2H, pyridyl-H, quinazolinyl-H), 8.78 (d, 1H, J = 4.8 Hz, pyridyl-H), 8.88 (s, 1H, pyridyl-H), 9.06 (d, 1H, J = 2.1 Hz, quinazolinyl-H), 10.66 (s, 1H, NH exchanged by D_2O), 11.73 (s, 1H, NH exchanged by D_2O). ^{13}C-NMR (100 MHz DMSO-d_6, ppm): δ 88.05, 119.22, 122.60, 123.67, 123.88, 124.30 (phenyl C-2, C-6), 129.09, 130.35, 135.40, 137.07, 138.40, 139.88, 141.27, 148.77, 153.16, 155.80, 163.98 (C=O), 167.71 (C=O). Mass (*m*/*z*, rel. abundance): 483 (M^+, 3.49%), 106.05 (100%). Anal. calcd for $C_{20}H_{14}IN_5O_2$ (483.26): C, 49.71; H, 2.92; N, 14.49. Found: C, 49.61; H, 2.66; N, 14.70.

*1-(6-Iodo-4-oxo-2-(pyridin-3-yl)quinazolin-3(4H)-yl)-3-phenylthiourea (**8e**).* Mp: 270 °C. Yield: 85%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3246 (NH), 3062 (CH aromatic), 1681 (C=O), 1635 (C=N), 1521–1483 (C=C), 1180 (C=S), 530 (C–I). ^{1}H-NMR 300 MHz (DMSO-d_6, D_2O, ppm): δ 7.05 (t, 1H, J = 6.9 Hz, phenyl-H), 7.38 (t, 2H, J = 6.9 Hz, phenyl-H), 7.61–7.70 (m, 3H, phenyl-2H, pyridyl-H), 7.95 (d, 1H, J = 8.70 Hz, quinazolinyl-H), 8.11 (s, 1H, quinazolinyl-H), 8.36 (d, 1H, J = 7.80 Hz, quinazolinyl-H), 8.46 (d, 1H, J = 8.70 Hz, pyridyl-H), 8.85 (d, 1H, J = 4.2 Hz, pyridyl-H), 9.22 (s, 1H, pyridyl-H), 10.83 (s, 1H, NH exchanged by D_2O), 11.48 (s, 1H, NH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 499 (M^+, 3.49%), 106.05 (100%). Anal. calcd for $C_{20}H_{14}IN_5OS$ (499.33): C, 48.11; H, 2.83; N, 14.03. Found: C, 47.99; H, 2.49; N, 14.33.

*1-(4-Chlorophenyl)-3-(6-iodo-4-oxo-2-(pyridin-3-yl)quinazolin-3(4H)-yl)urea (**8f**).* Mp: 251–253 °C. Yield%: 90%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3296, 3226 (2NH), 3099 (CH aromatic), 1683, 1670 (2C=O), 1645 (C=N), 1552–1448 (C=C), 723 (C–Cl), 530 (C–I). ^{1}H-NMR 300 MHz (DMSO-d_6, D_2O, ppm): δ 7.28 (d, J = 5.1 Hz, 2H, phenyl-H), 7.48–7.58 (m, 3H, phenyl-H, pyridyl-H), 7.98 (d, 1H, J = 2.10 Hz, quinazolinyl-H), 8.18–8.23 (m, 2H, quinazolinyl-H, pyridyl-H), 8.43 (s, 1H, quinazolinyl-H), 8.78 (dd, 1H, J = 3.00, 6.30 Hz, pyridyl-H), 9.06 (s, 1H, pyridyl-H), 10.69 (s, 1H, NH exchanged by D_2O), 11.74 (s, 1H, NH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 517 (M^+, 19.22%), 519 (M^+ + 2, 13.7%), 78 (100%). Anal. calcd for $C_{20}H_{13}ClIN_5O_2$ (517.71): C, 46.4; H, 2.53; N, 13.53. Found: C, 46.55; H, 2.80; N, 13.24.

1-(6-Iodo-4-oxo-2-(pyridin-4-yl)quinazolin-3(4*H*)-yl)-3-substituted urea/thiourea derivatives (8g–l). Compounds **8g–l** were prepared and purified by applying the same method described

for **8a–f** starting with **7b** and using the appropriate isocyanate or isothiocyanate.

*1-Ethyl-3-(6-iodo-4-oxo-2-(pyridin-4-yl)quinazolin-3(4H)-yl)thiourea (**8g**).* Mp: 229–231 °C. Yield: 83%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3321 (2NH), 3134 (CH aromatic), 2968, 2931 (CH_2, CH_3), 1683 (C=O), 1598 (C=N), 1512–1483 (C=C), 1213 (C=S), 530 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 1.04 (t, 3H, J = 7.04, CH_3CH_2), 3.43–3.5 (q, 2H, J = 7.00, CH_3CH_2), 7.79 (d, 2H, J = 6.00 Hz, pyridyl-H), 7.98 (dd, 1H, J = 1.88, 8.68 Hz, quinazolinyl-H), 8.21 (d, 2H, J = 8.68 Hz, quinazolinyl-H), 8.86 (d, 2H, J = 6.00 Hz, pyridyl-H), 10.74 (s, 1H, NH exchanged by D_2O), 11.85 (s, 1H, NH exchanged by D_2O). ^{13}C-NMR (100 MHz DMSO-d_6, ppm): δ 19.03 (CH_3), 39.05 (CH_2), 88.34, 121.32, 123.51, 123.86, 137.4, 138.26, 141.44, 141.57, 151.28, 163.76, 167.48 (C=O), 181.63 (C=S). Mass (*m*/*z*, rel. abundance): 451 (M^+, 0.12%), 75 (100%). Anal. calcd for $C_{16}H_{14}IN_5OS$ (451.28): C, 42.58; H, 3.13; N, 15.52. Found: C, 42.67; H, 3.33; N, 15.26.

*1-Cyclohexyl-3-(6-iodo-4-oxo-2-(pyridin-4-yl)quinazolin-3(4H)-yl)urea (**8h**).* Mp: 225–227 °C. Yield: 85%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3315 (br.) (2NH), 2924, 2850 (CH_2 aliphatic), 1687, 1660 (2C=O), 1647 (C=N), 1506 (C=C), 530 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 1.25–1.72 (m, 10H, cyclohexyl-5CH_2), 3.39–3.46 (m, 1H, cyclohexyl-CH), 7.79 (d, 2H, J = 6.00 Hz, pyridyl-H), 7.95 (dd, 1H, J = 1.92, 8.72 Hz, quinazolinyl-H), 8.14 (d, 1H, J = 1.92 Hz, quinazolinyl-H), 8.21 (d, 1H, J = 8.68 Hz, quinazolinyl-H), 8.82 (d, 2H, J = 6.00 Hz, pyridyl-H), 10.48 (s, 1H, NH exchanged by D_2O), 11.86 (s, 1H, NH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 489 (M^+, 0.24%), 56 (100%). Anal. calcd for $C_{20}H_{20}IN_5O_2$ (489.31): C, 49.09; H, 4.12; N, 14.31. Found: C, 49.16; H, 3.92; N, 14.56.

*1-Cyclohexyl-3-(6-iodo-4-oxo-2-(pyridin-3-yl)quinazolin-3(4H)-yl)thiourea (**8i**).* Mp: 254–256 °C. Yield: 70%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3325, 3230 (2NH), 2929, 2850 (CH_2 aliphatic), 1680 (C=O), 1640 (C=N), 1508 (C=C), 1116 (C=S), 530 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 0.98–1.22 (m, 5H, cyclohexyl), 1.53–1.75 (m, 5H, cyclohexyl), 4.07 (m, 1H, cyclohexyl-CH), 7.77 (d, J = 5.40 Hz, 2H, pyridyl-H), 7.95–8.09 (m, 3H, quinazolinyl-H), 8.82 (d, 2H, J = 5.40 Hz, pyridyl-H), 10.64 (s, 1H, NH exchanged by D_2O), 11.67 (s, 1H, NH exchanged by D_2O). Anal. calcd for $C_{20}H_{20}IN_5OS$ (505.38): C, 47.53; H, 3.99; N, 13.86. Found: C, 47.77; H, 4.08; N, 13.98.

*1-(6-Iodo-4-oxo-2-(pyridin-4-yl)quinazolin-3(4H)-yl)-3-phenylurea (**8j**).* Mp: 263–265 °C. Yield: 81% I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3304, 3211 (2NH), 3042 (CH aromatic), 1678, 1668 (2C=O), 1639 (C=N), 1558 (C=C), 530 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 6.97 (t, 1H, J = 7.32 Hz, phenyl-H), 7.26 (t, 2H, J = 7.92 Hz, phenyl-H), 7.46 (d, 2H, J = 7.92 Hz, phenyl-H), 7.95 (d, 2H, J = 5.76 Hz, pyridyl-H), 8.00 (dd, 1H, J = 1.6, 8.72 Hz quinazolinyl-H), 8.22 (d, 1H, J = 1.64 Hz, quinazolinyl-H), 8.26 (s, 1H, J = 8.72 Hz, quinazolinyl-H), 8.79 (d, 2H, J = 5.76 Hz, pyridyl-H), 10.7 (s, 1H, NH exchanged by D_2O), 11.88 (s, 1H, NH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 483 (M^+, 0.22%), 91 (100%). Anal. calcd for $C_{20}H_{14}IN_5O_2$ (483.26): C, 49.71; H, 2.92; N, 14.49. Found: C, 49.65; H, 2.98; N, 14.36.

*1-(6-Iodo-4-oxo-2-(pyridin-4-yl)quinazolin-3(4H)-yl)-3-phenylthiourea (**8k**).* Mp: 227–229 °C. Yield: 78%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3340, 3300 (NH), 3062 (CH aromatic), 1693 (C=O), 1614 (C=N), 1531–1481 (C=C), 1213 (C=S), 530 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 7.20 (t, 1H, J = 7.20 Hz, phenyl-H), 7.32–7.36 (m, 4H, phenyl-H), 7.40–7.55 (m, 1H, quinazolinyl-H), 7.75–7.77 (m, 2H, pyridyl-H), 7.98 (dd, 1H, J = 8.70 Hz, quinazolinyl-H), 8.23 (s, 1H, quinazolinyl-H), 8.80 (d, 2H, J = 6.30 Hz, pyridyl-H), 9.72 (s, 1H, NH exchanged by D_2O), 11.82 (s, 1H, NH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 499 (M^+, 1.55%), 364 (100%). Anal. calcd for $C_{20}H_{14}IN_5OS$ (499.33): C, 48.11; H, 2.83; N, 14.03. Found: C, 48.32; H, 2.81; N, 14.25.

*1-(4-Chlorophenyl)-3-(6-iodo-4-oxo-2-(pyridin-4-yl)quinazolin-3(4H)-yl)urea (**8l**).* Mp: 238–240 °C. Yield%: 92%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3280 (2NH), 3107 (CH aromatic), 1666, 1649 (2CO), 1595 (C=N), 1558–1444 (C=C), 688 (C–Cl), 530 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 7.31 (d, 2H, J = 8.52 Hz, phenyl-H), 7.50 (d, 2H, J = 8.52 Hz, phenyl-H), 7.78 (d, 2H, J = 4.84 Hz, pyridyl-H), 7.99 (d, H, J = 8.56 Hz, quinazolinyl-H), 8.22 (s, 1H, quinazolinyl-H), 8.26 (d, 1H, J = 8.56 Hz, quinazolinyl-H), 8.80 (d, 2H, J = 4.84 Hz, pyridyl-H), 10.72 (s, 1H, NH exchanged by D_2O), 11.87 (s, 1H, NH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 517 (M^+, 2.16%), 519 (M^+ + 2, 1.48%), 115 (100%). Anal. calcd for $C_{20}H_{13}ClIN_5O_2$ (517.71): C, 46.4; H, 2.53; N, 13.53. Found: C, 46.36; H, 2.82; N, 13.37.

N-(2-{[2-(4-Substituted benzylidene)hydrazinyl]carbonyl}-4-iodophenyl)pyridine-3-carboxamides (9a–g)

General method. The appropriate *p*-substituted benzaldehyde (2.40 mmol), the hydrazido derivative **7a** (0.72 g, 2 mmol) in absolute ethanol (10 mL) and in the presence of glacial acetic acid (1 mL) were refluxed for 5–8 h. The reaction mixture was left to cool; the obtained solid was filtered, washed with diethyl ether, dried and recrystallized from absolute ethanol to produce the pure compounds **9a–g**.

*N-(2-{[2-(4-Fluorobenzylidene)hydrazinyl]carbonyl}-4-iodophenyl)-pyridine-3-carboxamide (**9a**).* Mp: 237–239 °C. Yield: 65%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3309 (2NH), 3100 (CH aromatic), 2920 (C–H), 1687 (C=O), 1654 (C=O), 1597 (C=N), 1506 (C=C), 1234 (C–F), 530 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 7.32 (t, 2H, J = 8.76 Hz, fluorophenyl-H), 7.63 (dd, 1H, J = 4.84, 7.88 Hz, pyridyl-H), 7.82 (dd, 2H, J = 5.64, 8.76 Hz, fluorophenyl-H), 7.97 (dd, 1H, J = 1.8 Hz, 8.64 Hz, iodophenyl-H), 8.19–8.27 (m, 3H, iodophenyl-H, pyridyl-H), 8.44 (s, 1H, C–H), 8.81 (dd, 1H, J = 1.36, 4.76 Hz, pyridyl-H), 9.1 (d, 1H, J = 1.84 Hz, pyridyl-H), 11.78 (s, 1H, NH exchanged by D_2O), 12.56 (s, 1H, NH exchanged by D_2O). Anal. calcd for $C_{20}H_{14}FIN_4O_2$ (488.25): C, 49.20; H, 2.89; N, 11.47. Found: C, 49.29; H, 2.91; N, 11.62.

*N-(2-{[2-(4-Chlorobenzylidene)hydrazinyl]carbonyl}-4-iodophenyl)-pyridine-3-carboxamide (**9b**).* Mp: 242–244 °C. Yield: 66%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3199 (NH), 3103 (CH aromatic), 2924 (C–H), 1672, 1660 (2C=O), 1595 (C=N), 1508 (C=C), 705 (C–Cl), 514 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 7.54 (d, 2H, J = 8.44 Hz, chloro phenyl-H), 7.63 (dd, 1H, J = 4.84 Hz, 7.88 Hz, pyridyl-H), 7.78 (d, 2H, J = 8.44 Hz, chlorophenyl-H), 7.97 (dd, 1H, J = 1.80, 8.64 Hz, iodophenyl-H), 8.19–8.27 (m, 3H, iodophenyl-H, pyridyl-H), 8.43 (s, 1H, C–H), 8.81 (dd, 1H, J = 1.24, 4.76 Hz, pyridyl-H), 9.1 (d, 1H, J = 1.8 Hz, pyridyl-H), 11.75 (s, 1H, NH exchanged by D_2O), 12.20 (s, 1H, NH exchanged by D_2O). ^{13}C-NMR 100 MHz (DMSO-d_6, D_2O, ppm): δ 88.16, 124.21, 124.37, 129.23, 129.40, 129.47, 130.36, 133.38, 135.36, 135.42,

137.09, 138.76, 141.32, 148.33, 148.79, 153.14 (C–H), 163.77 (C=O), 163.82 (C=O). Mass (*m*/*z*, rel. abundance): 504 (M^+, 1.06%), 506 (M^+ + 2, 0.37%), 78 (100%). Anal. calcd for $C_{20}H_{14}ClIN_4O_2$ (504.71): C, 47.59; H, 2.80; N, 11.10. Found: C, 47.72; H, 2.78; N, 11.24.

*N-(2-{[2-(4-Bromobenzylidene)hydrazinyl]carbonyl}-4-iodophenyl)-pyridine-3-carboxamide (**9c**).* Mp: 243–245 °C. Yield: 75%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3197 (2NH), 3059 (CH aromatic), 2922 (C–H), 1672, 1660 (2C=O), 1593 (C=N), 1508 (C=C), 610 (C–Br), 520 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 7.63 (dd, 1H, J = 4.92, 7.96 Hz, pyridyl-H), 7.69 (m, 4H, bromophenyl-H), 7.97 (d, 1H, J = 1.88, 6.84 Hz, iodophenyl-H), 8.19–8.28 (m, 3H, iodophenyl-H, pyridyl-H), 8.41 (s, 1H, C–H), 8.81 (dd, 1H, J = 1.28, 3.48 Hz, pyridyl-H), 9.1 (d, 1H, J = 1.84 Hz, pyridyl-H), 11.75 (s, 1H, NH exchanged by D_2O), 12.20 (s, 1H, NH exchanged by D_2O). Anal. calcd for $C_{20}H_{14}BrIN_4O_2$ (549.16): C, 43.74; H, 2.57; N, 10.20. Found: C, 43.79; H, 2.53; N, 10.28.

*N-[2-({2-[4-(Dimethylamino)benzylidene]hydrazinyl}carbonyl)-4-iodophenyl]pyridine-3-carboxamide (**9d**).* Mp: 255–257 °C. Yield: 80%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3253 (2NH), 3194 (CH aromatic), 2987 (CH, CH_3), 1668, 1653 (2C=O), 1589 (C=N), 1510 (C=C), 510 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 2.99 (s, 6H, 2 × CH_3), 6.67 (d, 2H, J = 8.80 Hz, *N*,*N*-dimethylaminophenyl-H), 7.56 (d, 2H, J = 8.80 Hz, dimethylaminophenyl-H), 7.64 (dd, 1H, J = 4.88, 7.88 Hz, pyridyl-H), 7.95 (dd, 1H, J = 1.92, 8.76 Hz, iodophenyl-H), 8.2 (d, 1H, J = 1.92 Hz, iodophenyl-H), 8.26–8.31 (m, 2H, iodophenyl-H, pyridyl-H), 8.31 (s, 1H, C–H), 8.82 (dd, 1H, J = 1.44, 4.8 Hz, pyridyl-H), 9.11 (d, 1H, J = 1.88 Hz, pyridyl-H), 11.87 (s, 1H, NH exchanged by D_2O), 12.01 (s, 1H, NH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 513 (M^+, 1.44%), 163 (100%). Anal. calcd for $C_{22}H_{20}IN_5O_2$ (513.33): C, 51.47; H, 3.93; N, 13.64. Found: C, 51.53; H, 3.98; N, 13.81.

*N-[4-Iodo-2-({2-[4-(trifluoromethyl)benzylidene]hydrazinyl}carbonyl) phenyl]-pyridine-3-carboxamide (**9e**).* Mp: 245–247 °C. Yield: 72%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3427 (2NH), 3192 (CH aromatic), 2980 (C–H), 1681 (2C=O), 1595 (C=N), 1508 (C=C), 510 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 7.62 (dd, 1H, J = 3.00, 4.92 Hz, pyridyl-H), 7.82 (d, 2H, J = 8.28 Hz, trifluoromethyl phenyl-H), 7.95–7.99 (m, 3H, phenyl-H), 8.18–8.27 (m, 3H, phenyl-H, pyridyl-H), 8.50 (s, 1H, C–H), 8.81 (dd, 1H, J = 3.36, 4.76 Hz, pyridyl-H), 9.1 (s, J = 1.8 Hz, 1H, pyridyl-H), 11.69 (s, 1H, NH exchanged by D_2O), 12.31 (s, 1H, NH exchanged by D_2O). Anal. calcd for $C_{21}H_{14}F_3IN_4O_2$ (538.26): C, 46.86; H, 2.62; N, 10.41. Found: C, 46.94; H, 2.68; N, 10.54.

*N-(2-{[2-(4-Hydroxybenzylidene)hydrazinyl]carbonyl}-4-iodophenyl) pyridine-3-carboxamide (**9f**).* Mp: 273–275 °C. Yield: 79%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3238 (OH), 3161 (2 NH), 3080 (CH aromatic), 2924 (C–H), 1718, 1680 (2C=O), 1593 (C=N), 1496 (C=C), 510 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 6.85 (d, 2H, J = 8.44 Hz, hydroxyphenyl-H), 7.59 (d, 2H, J = 8.44 Hz, hydroxyphenyl-H), 7.63 (dd, 1H, J = 2.92, 4.96 Hz, pyridyl-H), 7.95 (dd, 1H, J = 1.44, 8.76 Hz, iodophenyl-H), 8.20 (d, 1H, J = 1.56 Hz, iodophenyl-H), 8.26–8.28 (m, 2H, iodophenyl-H, pyridyl-H), 8.34 (s, 1H, C–H), 8.82 (dd, 1H, J = 3.72, 4.72 Hz, pyridyl-H), 9.10 (d, 1H, J = 1.84 Hz, pyridyl-H), 10.00 (s, 1H, OH exchanged by D_2O), 11.93 (s, 1H, NH exchanged by D_2O), 11.96 (s, 1H, NH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 486 (M^+, 1.8%), 67 (100%). Anal. calcd for $C_{20}H_{15}IN_4O_3$ (486.26): C, 49.40; H, 3.11; N, 11.52. Found: C, 49.56; H, 3.16; N, 11.63.

*N-(4-Iodo-2-{[2-(4-nitrobenzylidene)hydrazinyl]carbonyl}phenyl) pyridine-3-carboxamide (**9g**).* Mp: 298–300 °C. Yield: 88%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3329 (2NH), 3103 (CH aromatic), 2949 (C–H), 1683, 1660 (2C=O), 1598 (C=N), 1577 (C=C), 1517 (C–NO_2), 510 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 7.63 (dd, 1H, J = 4.88, 7.08 Hz, pyridyl-H), 7.97–8.07 (m, 3H, phenyl-H), 8.16–8.32 (m, 5H, phenyl-H, pyridyl), 8.53 (s, 1H, C–H), 8.80 (d, 1H, J = 3.56 Hz, pyridyl-H), 9.1 (s, 1H, pyridyl-H), 11.65 (s, 1H, NH exchanged by D_2O), 12.41 (s, 1H, NH exchanged by D_2O). Anal. calcd for $C_{20}H_{14}IN_5O_4$ (515.26): C, 46.62; H, 2.74; N, 13.59. Found: C, 46.80; H, 2.71; N, 13.68.

***N*-(2-{[2-(4-Substituted benzylidene)hydrazinyl]carbonyl}-4-iodophenyl)pyridine-4-carboxamides (9h–n).** Compounds **9h–n** were prepared according to the last mentioned procedure using **7b** as a starting material and were recrystallized from absolute ethanol.

*N-(2-{[2-(4-Fluorobenzylidene)hydrazinyl]carbonyl}-4-iodophenyl) pyridine-4-carboxamide (**9h**).* Mp: 288–290 °C. Yield: 59%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3296 (NH), 3122 (CH aromatic), 2820 (C–H), 1683 (C=O), 1630 (C=N), 1508 (C=C), 1232 (C–F), 532 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 7.37 (t, 2H, J = 8.68 Hz, fluorophenyl-H), 7.61 (d, 1H, J = 8.56 Hz, iodophenyl-H), 7.67 (d, 2H, J = 5.92 Hz, pyridyl-H), 7.79 (d, 2H, J = 3.12 Hz, fluorophenyl-H), 8.22 (dd, 1H, J = 8.72 Hz, iodophenyl-H), 8.52 (d, 1H, J = 1.92 Hz, iodophenyl-H), 8.69 (d, 2H, J = 5.92 Hz, pyridyl-H), 9.08 (s, 1H, C–H), 11.70 (s, 1H, NH exchanged by D_2O), 12.20 (s, 1H, NH exchanged by D_2O). Anal. calcd for $C_{20}H_{14}FIN_4O_2$ (488.25): C, 49.20; H, 2.89; N, 11.47. Found: C, 49.29; H, 2.92; N, 11.59.

*N-(2-{[2-(4-Chlorobenzylidene)hydrazinyl]carbonyl}-4-iodophenyl) pyridine-3-carboxamide (**9i**).* Mp: 262–264 °C. Yield: 83%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3446 (NH), 3113 (CH aromatic), 2843 (C–H), 1680 (2C=O), 1587 (C=N), 1506–1442 (C=C), 750 (C–Cl), 520 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 7.54 (d, 2H, J = 8.40 Hz, chloro phenyl-H), 7.78 (d, 2H, J = 8.40 Hz, chloro phenyl-H), 7.82 (d, 2H, J = 5.88 Hz, pyridyl-H), 7.99 (dd, 1H, J = 1.64, 8.72 Hz, iodophenyl-H), 8.21 (d, 1H, J = 1.60 Hz, iodophenyl-H), 8.24 (d, 1H, J = 8.72 Hz, iodophenyl-H), 8.44 (s, 1H, C–H), 8.84 (d, 2H, J = 5.88 Hz, pyridyl-H), 11.88 (s, 1H, NH exchanged by D_2O), 12.22 (s, 1H, NH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 504 (M^+, 0.23%), 506 (M^+ + 2, 0.13%), 78 (100%). Anal. calcd for $C_{20}H_{14}ClIN_4O_2$ (504.71): C, 47.59; H, 2.80; N, 11.10. Found: C, 47.67; H, 2.82; N, 11.23.

*N-(2-{[2-(4-Bromobenzylidene)hydrazinyl]carbonyl}-4-iodophenyl) pyridine-4-carboxamide (**9j**).* Mp: 283–285 °C. Yield: 70%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3423 (NH), 3076 (CH aromatic), 2843 (C–H), 1685 (2C=O), 1577 (C=N), 1506 (C=C), 526 (C–Br), 514 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 7.67–7.72 (m, 4H, bromophenyl-H), 7.82 (d, 2H, J = 5.80 Hz, pyridyl-H), 7.98 (dd, 1H, J = 1.60, 8.72 Hz, iodophenyl-H), 8.21 (d, 1H, J = 1.60 Hz, iodophenyl-H), 8.24 (d, 1H, J = 8.76 Hz, iodophenyl-H), 8.42 (s, 1H, C–H), 8.84 (d, 2H, J = 5.80 Hz, pyridyl-H), 11.87 (s, 1H, NH exchanged by D_2O), 12.22 (s, 1H, NH exchanged by D_2O). Anal. calcd for $C_{20}H_{14}BrIN_4O_2$ (549.16): C, 43.74; H, 2.57; N, 10.20. Found: C, 43.85; H, 2.53; N, 10.34.

*N-[2-({2-[4-(Dimethylamino)benzylidene]hydrazinyl}carbonyl)-4-iodophenyl]-pyridine-4-carboxamide (**9k**).* Mp: 228–230 °C. Yield: 80%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3408 (NH), 3159 (CH aromatic), 2880 (CH, CH_3(s)), 1672 (2C=O), 1610 (C=N), 1508 (C=C), 514 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 3.01 (s, 6H, 2 × CH_3), 6.77 (d, 2H, J = 8.92 Hz, aminophenyl-H), 7.56 (d, 2H, J = 8.92 Hz, aminophenyl-H), 7.83 (d, 2H, J = 5.96 Hz, pyridyl-H), 7.96 (dd, 1H, J = 1.80, 8.76 Hz, iodophenyl-H), 8.22 (d, 1H, J = 1.84 Hz, iodophenyl-H), 8.31 (d, 1H, J = 1.6 Hz, iodophenyl-H), 8.33 (s, 1H, C–H), 8.85 (d, 2H, J = 5.96 Hz, pyridyl-H), 11.89 (s, 1H, NH exchanged by D_2O), 12.43 (s, 1H, NH exchanged by D_2O). ^{13}C-NMR 100 MHz (DMSO-d_6, D_2O, ppm): δ 56.49 (2 × CH_3 carbon), 88.25, 112.24, 121.36, 121.48, 123.69, 124.23, 129.24, 136.95, 138.78, 141.18, 141.70, 150.88 (C–H), 151.28, 152.25, 163.25 (C=O), 163.48 (C=O). Anal. calcd for $C_{22}H_{20}IN_5O_2$ (513.33): C, 51.47; H, 3.93; N, 13.64. Found: C, 51.53; H, 3.98; N, 13.81.

*N-[4-Iodo-2-({2-[4-(trifluoromethyl)benzylidene]hydrazinyl}carbonyl)phenyl]-pyridine-4-carboxamide (**9l**).* Mp: 238–240 °C. Yield: 67%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3412 (NH), 3211 (CH aromatic), 2840 (C–H), 1678 (C=O), 1651 (C=O), 1589 (C=N), 1508–1444 (C=C), 540 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 7.82–7.84 (m, 4H, trifluorophenyl-H), 7.97 (d, 2H, J = 7.88 Hz, pyridyl-H), 8.00 (d, 1H, J = 1.64, iodophenyl-H), 8.21–8.23 (m, 2H, iodophenyl-H), 8.51 (s, 1H, C–H), 8.84 (d, 2H, J = 7.88 Hz, pyridyl-H), 11.82 (s, 1H, NH exchanged by D_2O), 12.33 (s, 1H, NH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 538 (M^+, 0.44%), 106 (100%). Anal. calcd for $C_{21}H_{14}F_3IN_4O_2$ (538.26): C, 46.86; H, 2.62; N, 10.41. Found: C, 46.95; H, 2.61; N, 10.50.

*N-(2-{[2-(4-Hydroxybenzylidene)hydrazinyl]carbonyl}-4-iodophenyl)pyridine-4-carboxamide (**9m**).* Mp: 297–299 °C. Yield: 90%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3176 (OH), 3111 (NH), 3057 (CH aromatic), 2868 (C–H), 1685 (C=O), 1643 (C=O), 1610 (C=N), 1512 (C=C), 536 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 6.86 (d, 2H, J = 8.44 Hz, hydroxyphenyl-H), 7.59 (d, 2H, J = 8.44 Hz, hydroxyphenyl-H), 7.82 (d, 2H, J = 5.68 Hz, pyridyl-H), 7.96 (d, 1H, J = 8.64 Hz, iodophenyl-H), 8.21 (d, 1H, J = 1.40 Hz, iodophenyl-H), 8.30 (d, 1H, J = 8.72 Hz, iodophenyl-H), 8.35 (s, 1H, C–H), 8.84 (d, 2H, J = 5.68 Hz, pyridyl-H), 10.01 (s, 1H, OH exchanged by D_2O), 11.98 (s, 1H, NH exchanged by D_2O), 12.07 (s, 1H, NH exchanged by D_2O). Anal. calcd for $C_{20}H_{15}IN_4O_3$ (486.26): C, 49.40; H, 3.11; N, 11.52. Found: C, 49.48; H, 3.14; N, 11.58.

*N-(4-Iodo-2-{[2-(4-nitrobenzylidene)hydrazinyl]carbphenyl)pyridine-4-carboxamide (**9n**).* Mp: 298–300 °C. Yield: 88%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3487 (NH), 3109 (CH aromatic), 2837 (C–H), 1689 (C=O), 1645 (C=O), 1635 (C=N), 1555 (C–NO_2), 1514 (C=C), 530 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 7.82 (d, 2H, J = 5.8 Hz, pyridyl-H), 8.00–8.02 (m, 3H, phenyl-H), 8.19 (d, 1H, J = 8.7 Hz, iodophenyl-H), 8.21 (d, 1H, J = 1.64 Hz, iodophenyl-H), 8.32 (d, 2H, J = 8.44 Hz, nitrophenyl-H), 8.53 (s, 1H, C–H), 8.84 (d, 2H, J = 5.8 Hz, pyridyl-H), 11.76 (s, 1H, NH exchanged by D_2O), 12.41 (s, 1H, NH exchanged by D_2O). Anal. calcd for $C_{20}H_{14}IN_5O_4$ (515.26): C, 46.62; H, 2.74; N, 13.59. Found: C, 46.80; H, 2.71; N, 13.68.

3-[(4-Substituted benzylidene)amino]-6-iodo-2-(pyridin-3-yl) quinazolin-4(3*H*)-one (10a–d). A mixture containing equimolar amounts of sodium ethoxide and the diamide **9a**, **9b**, **9c** or **9g** (1 mmol) was heated under reflux for 3 h. The reaction mixture was cooled to room temperature. The separated solid was filtered, washed with water and recrystallized from ethanol.

*3-[(4-Fluorobenzylidene)amino]-6-iodo-2-(pyridin-3-yl)quinazolin-4(3H)-one (**10a**).* Mp: 337–339 °C. Yield: 80%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3190 (CH aromatic), 2922 (C–H), 1681 (C=O), 1593 (C=N), 1506 (C=C), 1226 (C–F), 530 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, ppm): δ 7.23 (t, 2H, J = 8.64 Hz, fluorophenyl-H), 7.59 (d, 2H, J = 7.44 Hz, fluorophenyl-H), 7.64 (d, 1H, J = 8.68 Hz, quinazolinyl-H), 7.70 (t, 1H, J = 7.00 Hz, pyridyl-H), 8.18 (s, 1H, C–H), 8.32 (d, 1H, J = 7.76 Hz, quinazolinyl-H), 8.47 (d, 1H, J = 8.64 Hz, pyridyl-H), 8.51 (s, 1H, quinazolinyl-H), 8.77 (d, 1H, J = 3.76 Hz, pyridyl), 9.17 (s, 1H, pyridyl). Mass (*m*/*z*, rel. abundance): 470 (M^+, 9.28%), 78 (100%). Anal. calcd for $C_{20}H_{12}FIN_4O$ (470.24): C, 51.08; H, 2.57; N, 11.91. Found: C, 51.19; H, 2.55; N, 12.04.

*3-[(4-Chlorobenzylidene)amino]-6-iodo-2-(pyridin-3-yl)quinazolin-4(3H)-one (**10b**).* Mp: 238–340 °C. Yield: 77%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3290 (CH aromatic), 2922 (C–H), 1645 (C=O), 1577 (C=N), 1504 (C=C), 750 (C–Cl), 520 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, ppm): δ 7.44 (d, 2H, J = 7.76 Hz, chloro phenyl-H), 7.82 (d, 2H, J = 8.54 Hz, chloro phenyl-H), 7.89 (d, 1H, J = 5.88 Hz, pyridyl-H), 8.20 (dd, 1H, J = 1.58, 8.70 Hz, quinazlinyl-H), 8.24–8.35 (m, 3H, quinazolinyl-H, pyridyl-H), 8.44 (d, 1H, J = 8.72 Hz, quinazolinyl-H), 8.65 (s, 1H, C–H), 8.84 (d, 1H, J = 6.00 Hz, pyridyl-H). Anal. calcd for $C_{20}H_{12}ClIN_4O$ (486.69): C, 49.36; H, 2.49; N, 11.51. Found: C, 49.08; H, 2.60; N, 11.77.

*3-[(4-Bromobenzylidene)amino]-6-iodo-2-(pyridin-3-yl)quinazolin-4(3H)-one (**10c**).* Mp: 335–337 °C. Yield: 81%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3284 (CH aromatic), 2951 (C–H), 1678 (C=O), 1587 (C=N), 1506 (C=C), 526 (C–Br), 514 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, ppm): δ 7.56–7.62 (m, 4H, bromophenyl-H), 7.65 (d, 2H, J = 2.76 Hz, pyridyl-H, quinazolinyl-H), 8.13 (s, 1H, C–H), 8.32 (d, 1H, J = 7.84 Hz, quinazolinyl-H), 8.47 (d, 2H, J = 8.72 Hz, quinazolinyl-H, pyridyl-H), 8.50 (s, 1H, pyridyl-H), 8.77 (d, 1H, J = 4.44 Hz, pyridyl-H). Mass (*m*/*z*, rel. abundance): 531 (M^+, 19.59%), 532 (M^+ + 1, 13.21%), 78 (100%). Anal. calcd for $C_{20}H_{12}BrIN_4O$ (531.14): C, 45.23; H, 2.28; N, 10.55. Found: C, 45.31; H, 2.26; N, 10.63.

*6-Iodo-3-((4-nitrobenzylidene)amino)-2-(pyridin-3-yl)quinazolin-4(3H)-one (**10d**).* Mp: 315–317 °C. Yield: 70%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3064 (CH aromatic), 2954 (CH), 1654 (C=O), 1629 (C=N), 1512 (C–NO_2), 1506 (C=C), 514 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, ppm): δ 7.60–7.73 (m, 4H, nitrophenyl-H), 8.22 (d, 2H, J = 8.28 Hz, pyridyl-H, quinazolinyl-H), 8.30–8.34 (m, 2H, pyridyl-H, quinazolinyl-H), 8.46 (d, 1H, J = 7.36 Hz, quinazolinyl-H), 8.53 (s, 1H, C–H), 8.80 (d, 1H, J = 4.64 Hz, pyridyl-H), 9.17 (s, 1H, pyridyl-H). Mass (*m*/*z*, rel. abundance): 497 (M^+, 37.31%), 78 (100%). Anal. calcd for $C_{20}H_{12}IN_5O_3$ (497.25): C, 48.31; H, 2.43; N, 14.08. Found: C, 48.50; H, 2.46; N, 14.17.

3-[(4-Substituted benzylidene)amino]-6-iodo-2-(pyridin-4-yl) quinazolin-4(3*H*)-one (10e–h). Compounds **10e–h** were similarly prepared and purified adopting the method described for compounds **10a–f**.

*3-[(4-Fluorobenzylidene)amino]-6-iodo-2-(pyridin-4-yl)quinazolin-4(3H)-one (**10e**).* Mp: 304–306 °C. Yield: 59%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3111 (CH aromatic), 2921 (C–H), 1645 (C=O), 1598

(C=N), 1506 (C=C), 1230 (C–F), 536 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, ppm): δ 7.32 (t, 2H, J = 7.60 Hz, phenyl-H), 7.82 (d, 2H, J = 7.60 Hz, phenyl-H), 7.98 (d, 2H, J = 8.76 Hz, quinazolinyl-H), 8.21 (d, 2H, J = 5.44 Hz, pyridyl-H), 8.21–8.26 (m, 2H, quinazolinyl-H), 8.45 (s, 1H, C–H), 8.84 (d, 2H, J = 5.44 Hz, pyridyl-H). Mass (m/z, rel. abundance): 470 (M^+, 0.46%), 75 (100%). Anal. calcd for $C_{20}H_{12}FIN_4O$ (470.24): C, 51.08; H, 2.57; N, 11.91. Found: C, 51.22; H, 2.62; N, 12.07.

*3-[(4-Chlorobenzylidene)amino]-6-iodo-2-(pyridin-4-yl)quinazolin-4(3H)-one (**10f**).* Mp: 331–333 °C. Yield: 65%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3111 (CH aromatic), 2920 (C–H), 1654 (C=O), 1597 (C=N), 1508 (C=C), 750 (C–Cl), 520 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, ppm): δ 7.44 (d, 2H, J = 8.28 Hz, chloro phenyl-H), 7.64 (dd, 1H, J = 1.76, 8.76 Hz, quinazolinyl-H), 7.67 (d, 2H, J = 8.40 Hz, chloro phenyl-H), 7.88 (d, 2H, J = 5.44 Hz, pyridyl-H), 8.16 (s, 1H, C–H), 8.45 (d, 1H, J = 1.60 Hz, quinazolinyl-H), 8.50 (d, 1H, J = 8.72 Hz, quinazolinyl-H), 8.78 (d, 2H, J = 5.44 Hz, pyridyl-H). Anal. calcd for $C_{20}H_{12}ClIN_4O$ (486.69): C, 49.36; H, 2.49; N, 11.51. Found: C, 49.43; H, 2.54; N, 11.67.

*3-[(4-Bromobenzylidene)amino]-6-iodo-2-(pyridin-4-yl)quinazolin-4(3H)-one (**10g**).* Mp: 327–329 °C. Yield: 72%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3203 (CH aromatic), 2968 (C–H), 1649 (2C=O), 1577 (C=N), 1506 (C=C), 526 (C–Br), 512 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, ppm): δ 7.57–7.65 (m, 5H, bromophenyl-H, quinazolinyl-H), 7.88 (d, 2H, J = 5.84 Hz, pyridyl-H), 8.14 (d, 1H, J = 3.60 Hz, quinazolinyl-H), 8.46 (d, 1H, J = 8.60 Hz, quinazolinyl-H), 8.51 (s, 1H, C–H), 8.79 (d, 2H, J = 5.84 Hz, pyridyl-H). Anal. calcd for $C_{20}H_{12}BrIN_4O$ (531.14): C, 45.23; H, 2.28; N, 10.55. Found: C, 45.34; H, 2.31; N, 10.73.

*6-Iodo-3-((4-nitrobenzylidene)amino)-2-(pyridin-4-yl)quinazolin-4(3H)-one (**10h**).* Mp: 333 °C. Yield: 84%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3070 (CH aromatic), 2960 (CH), 1657 (C=O), 1610 (C=N), 1518 (C–NO_2), 1508 (C=C), 514 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, ppm): δ 7.68 (d, 1H, J = 8.36 Hz, quinazolinyl-H), 7.88–7.90 (m, 4H, nitrophenyl-H), 8.20 (d, 2H, J = 5.28 Hz, pyridyl-H), 8.29 (s, 1H, C–H), 8.47 (d, 1H, J = 8.40 Hz, quinazolinyl-H), 8.53 (s, 1H, quinazolinyl-H), 8.82 (d, 2H, J = 5.28 Hz, pyridyl-H). Mass (m/z, rel. abundance): 497 (M^+, 1.4%), 59 (100%). Calcd for $C_{20}H_{12}IN_5O_3$ (497.25): C, 48.31; H, 2.43; N, 14.08. Found: C, 48.39; H, 2.41; N, 14.19.

***N*-(2-{[2-(Chloroacetyl)hydrazinyl]carbonyl}-4-iodophenyl) pyridine-3-carboxamide (11a).** The hydrazido derivative **7a** (10 mmol, 3.64 g) and chloroacetyl chloride (15 mmol, 1.69 g) were stirred overnight at room temperature in dimethylformamide (10 mL). The reaction mixture was poured onto ice-cold water and the separated solid was collected, dried and recrystallized from methanol. Mp: 276–278 °C. Yield: 83% I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3479 (NH), 3176 (CH aromatic), 2953 (CH_2), 1707, 1687 (3C=O), 1651 (C=N), 1516 (C=C), 785 (C–Cl), 524 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 4.24 (s, 2H, CH_2), 7.62 (dd, 1H, J = 3.64, 7.88 Hz, pyridyl-H), 7.98 (dd, 1H, J = 1.72, 8.76 Hz, phenyl-H), 8.19 (d, 1H, J = 1.76 Hz, phenyl-H), 8.24 (d, 1H, J = 7.96 Hz, pyridyl-H), 8.29 (d, 1H, J = 8.76 Hz, phenyl-H), 8.80 (dd, 1H, J = 1.04, 4.68 Hz, pyridyl-H), 9.1 (d, 1H, J = 1.72 Hz, pyridyl-H), 10.56 (s, 1H, NH exchanged by D_2O), 10.98 (s, 1H, NH exchanged by D_2O), 11.81 (s, 1H, NH exchanged by D_2O). Anal. calcd for $C_{15}H_{12}ClIN_4O_3$ (458.64): C, 39.28; H, 2.64; N, 12.22. Found: C, 39.41; H, 2.68; N, 12.38.

***N*-(2-{[2-(Chloroacetyl)hydrazinyl]carbonyl}-4-iodophenyl) pyridine-4-carboxamide (11b).** This compound was prepared and purified as per the previously mentioned procedure, using the hydrazido derivative **7b**. Mp: 289–291 °C. Yield: 80%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3468, 3448 (NHs), 3178 (CH aromatic), 2974 (CH aliphatic), 1695, 1685 (3C=O), 1631 (C=N), 1508–1436 (C=C), 524 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 4.24 (s, 2H, CH_2), 7.79 (d, 2H, J = 5.48 Hz, pyridyl-H), 7.99 (d, 1H, J = 8.68 Hz, phenyl), 8.20 (s, 1H, phenyl), 8.30 (d, 1H, J = 8.76 Hz, phenyl-H), 8.83 (dd, 2H, J = 5.48 Hz, pyridyl-H), 10.58 (s, 1H, NH exchanged by D_2O), 11.00 (s, 1H, NH exchanged by D_2O), 11.89 (s, 1H, NH exchanged by D_2O). Mass (m/z, rel. abundance): 460 (M^+ + 2, 1.10%), 458 (M^+, 2.37%), 56 (100%). Anal. calcd for $C_{15}H_{12}ClIN_4O_3$ (458.64): C, 39.28; H, 2.64; N, 12.22. Found: C, 39.38; H, 2.68; N, 12.28.

***N*-(4-Iodo-2-{[2-(substituted acetyl)hydrazinyl]carbonyl} phenyl)pyridine-3-carboxamide (12a–d).** Equimolar amounts of chloroacetyl hydrazido derivative **11a** (0.45 g, 1 mmol) and the appropriate secondary amine (1 mmol) in absolute ethanol (15 mL) containing anhydrous potassium carbonate (1.5 mmol, 0.2 g), were refluxed for 12 h. The reaction mixture was filtered while hot and the clear solution was evaporated under reduced pressure. The remaining residue was collected, washed with water and recrystallized from ethanol affording the corresponding derivatives **12a–d**.

*N-(4-Iodo-2-{[2-(piperidin-1-ylacetyl)hydrazinyl]carbonyl}phenyl) pyridine-3-carboxamide (**12a**).* Mp: 200–202 °C. Yield: 55%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3419 (NHs), 3132 (CH aromatic), 2941 (CH_2), 1670 (C=O), 1653 (C=N), 1541 (C=C), 516 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 1.39 (d, J = 4.44 Hz, 2H, piperidinyl-H), 1.55 (t, J = 5.36 Hz, 4H, piperidinyl-H), 3.09 (s, 2H, CH_2), 3.34 (s, 4H, piperidinyl-H), 7.60 (dd, 1H, J = 4.84, 7.92 Hz, pyridyl-H), 7.95 (dd, 1H, J = 1.48, 8.72 Hz, phenyl-H), 8.20 (d, 1H, J = 1.68 Hz, phenyl-H), 8.30 (d, 1H, J = 7.60 Hz, pyridyl-H), 8.36 (d, 1H, J = 8.76 Hz, phenyl-H), 8.79 (dd, 1H, J = 1.04, 4.72 Hz, pyridyl-H), 9.07 (d, 1H, J = 1.64 Hz, pyridyl-H), 9.93 (s, 1H, NH exchanged by D_2O), 10.74 (s, 1H, NH exchanged by D_2O), 12.07 (s, 1H, NH exchanged by D_2O). Anal. calcd for $C_{20}H_{22}IN_5O_3$ (507.32): C, 47.35; H, 4.37; N, 13.80. Found: C, 47.54; H, 4.44; N, 13.96.

*N-(4-Iodo-2-{[2-(morpholin-4-ylacetyl)hydrazinyl]carbonyl}phenyl) pyridine-3-carboxamide (**12b**).* Mp: 268–270 °C. Yield: 53%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3251 (NHs), 3182 (CH aromatic), 2856 (CH_2), 1700, 1681 (3C=O), 1641 (C=N), 1556 (C=C), 514 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 2.52 (t, 4H, J = 4.04 Hz, morpholinyl-H), 3.11 (s, 2H, CH_2), 3.63 (t, 4H, J = 4.52 Hz, morpholinyl-H), 7.61 (dd, 1H, J = 4.80, 7.88 Hz, pyridyl-H), 7.97 (dd, 1H, J = 1.73, 8.80 Hz, phenyl-H), 8.18 (d, 1H, J = 1.44 Hz, phenyl-H), 8.25 (d, 1H, J = 7.88 Hz, pyridyl-H), 8.35 (d, 1H, J = 8.76 Hz, phenyl-H), 8.81 (dd, 1H, J = 1.12, 4.68 Hz, pyridyl-H), 9.06 (d, 1H, J = 1.76 Hz, pyridyl-H), 9.98 (s, 1H, NH exchanged by D_2O), 10.74 (s, 1H, NH exchanged by D_2O), 11.90 (s, 1H, NH exchanged by D_2O). Mass (m/z, rel. abundance): 509 (M^+, 1.04%), 60 (100%). Anal. calcd for $C_{19}H_{20}IN_5O_4$ (509.30): C, 44.81; H, 3.96; N, 13.75. Found: C, 44.89; H, 3.99; N, 13.89.

*Ethyl 4-({N′[(5-iodo-2-pyridine-3-amido)benzoyl]hydrazinecarbonyl} methyl)-piperazine-carboxylate (**12c**).* Mp: 237–239 °C. Yield: 78%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3244 (NHs), 3111 (CH aromatic), 2927 (CH_2 aliphatic), 1701, 1681, 1654 (3C=O), 1597 (C=N), 1508 (C=C), 516 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 1.18 (t, 3H, J = 7.08 Hz, CH_3CH_2), 3.15 (s, 2H, CH_2), 3.41 (s, 8H, piperazinyl-H), 4.04 (q, 2H, J = 7.04 Hz, CH_3CH_2), 7.61 (dd, 1H, J = 4.84, 7.84 Hz, pyridyl-H), 7.97 (dd, 1H, J = 1.68, 8.76 Hz, phenyl-H), 8.18 (d, 1H, J = 1.76 Hz, phenyl-H), 8.24 (d, 1H, J = 7.96 Hz, pyridyl-H), 8.35 (d, 1H, J = 8.80 Hz, phenyl-H), 8.80 (dd, 1H, J = 3.56, 4.76 Hz, pyridyl-H), 9.06 (d, 1H, J = 1.68 Hz, pyridyl-H), 10.01 (s, 1H, NH exchanged by D_2O), 10.80 (s, 1H, NH exchanged by D_2O), 11.89 (s, 1H, NH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 580 (M^+, 0.17%), 165 (100%). Anal. calcd for $C_{22}H_{25}IN_6O_5$ (580.38): C, 45.53; H, 4.34; N, 14.48. Found: C, 45.58; H, 4.36; N, 15.55.

*N-(4-Iodo-2-{[2-(4-phenylpiperazin-1-ylacetyl)hydrazinyl]carbonyl} phenyl)pyridine-3-carboxamide (**12d**).* Mp: 213–215 °C. Yield: 62%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3219 (NHs), 3188 (CH aromatic), 2823 (CH aliphatic), 1683, 1658 (3C=O), 1597 (C=N), 1506–1452 (C=C), 514 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 2.51 (t, 4H, J = 1.68 Hz, piperazinyl), 2.69 (s, 4H, piperazinyl), 3.19 (s, 2H, CH_2), 6.78 (t, 1H, J = 7.2 Hz, phenyl-H), 6.95 (d, 2H, J = 7.44 Hz, phenyl-H), 7.22 (t, 2H, J = 7.44 Hz, phenyl-H), 7.60 (dd, 1H, J = 4.84, 7.88 Hz, pyridyl-H), 7.97 (dd, 1H, J = 1.80, 8.72 Hz, iodophenyl-H), 8.19 (d, 1H, J = 1.88 Hz, iodophenyl-H), 8.25 (d, 1H, J = 7.96 Hz, pyridyl-H), 8.35 (d, 1H, J = 8.76 Hz, iodophenyl-H), 8.80 (dd, 1H, J = 1.32, 4.76 Hz, pyridyl-H), 9.07 (d, 1H, J = 1.80 Hz, pyridyl-H), 10.01 (s, 1H, NH exchanged by D_2O), 10.82 (s, 1H, NH exchanged by D_2O), 11.90 (s, 1H, NH exchanged by D_2O). Anal. calcd for $C_{25}H_{25}IN_6O_3$ (584.41): C, 51.38; H, 4.31; N, 14.38. Found: C, 51.54; N, 4.36; N, 14.48.

***N*-(4-Iodo-2-{[2-(substituted acetyl)hydrazinyl]carbonyl} phenyl)pyridine-4-carboxamide (12e–h).** Derivatives **12e–h** were prepared by applying the procedure used for the preparation of the nicotinamido analogues **12a–d** and were recrystallized from ethanol.

*N-(4-Iodo-2-{[2-(piperidin-1-ylacetyl)hydrazinyl]carbonyl}phenyl) pyridine-4-carboxamide (**12e**).* Mp: 204–206 °C. Yield: 45%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3412 (NHs), 3118 (CH aromatic), 2926 (CH_2 aliphatic), 1668 (3C=O), 1629 (C=N), 1577 (C=C), 518 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 1.40 (s, 2H, piperidinyl-H), 1.57 (s, 4H, piperidinyl-H), 3.08 (s, 2H, CH_2), 3.32 (s, 4H, piperidinyl-H), 7.83 (d, 2H, J = 5.68 Hz, pyridyl-H), 7.96 (d, 1H, J = 8.92 Hz, phenyl-H), 8.22 (s, 1H, phenyl-H), 7.36 (d, 1H, J = 8.84 Hz, phenyl-H), 8.82 (d, 2H, J = 5.68 Hz, pyridyl-H), 9.93 (s, 1H, NH exchanged by D_2O), 10.78 (s, 1H, NH exchanged by D_2O), 12.02 (s, 1H, NH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 507 (M^+, 1.32%), 85 (100%). Anal. calcd for $C_{20}H_{22}IN_5O_3$ (507.32): C, 47.35; H, 4.37; N, 13.80. Found: C, 47.50; H, 4.39; N, 13.89.

*N-(4-Iodo-2-{[2-(morpholin-4-ylacetyl)hydrazinyl]carbonyl}phenyl) yridine-4-carboxamide (**12f**).* Mp: 229–231 °C. Yield: 70%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3444 (NHs), 3120 (CH aromatic), 2927 (CH_2 aliphatic), 1697, 1681, 1651 (3C=O), 1598 (C=N), 1510–1440 (C=C), 520 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 2.51 (t, 4H, J = 1.72 Hz, morpholinyl-H), 3.12 (s, 2H, CH_2), 3.63 (t, 4H, J = 4.52 Hz, morpholinyl-H), 7.79 (d, 2H, J = 5.96 Hz, pyridyl-H), 7.98 (dd, 1H, J = 1.92, 8.76 Hz, phenyl-H), 8.20 (d, 1H, J = 1.96 Hz, phenyl-H), 8.35 (d, 1H, J = 8.76 Hz, phenyl-H), 8.82 (dd, 2H, J = 1.40, 4.52 Hz, pyridyl-H), 9.99 (s, 1H, NH exchanged by D_2O), 10.80 (s, 1H, NH exchanged by D_2O), 11.96 (s, 1H, NH exchanged by D_2O). Anal. calcd for $C_{19}H_{20}IN_5O_4$ (509.30): C, 44.81; H, 3.96; N, 13.75. Found: C, 44.89; H, 3.98; N, 13.79.

*Ethyl 4-({N′[(5-iodo-2-pyridine-4-amido)benzoyl]hydrazinecarbonyl} methyl)-piperazinecarboxylate (**12g**).* Mp: 176–178 °C. Yield: 60%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3479, 3419 (NHs), 3228 (CH aromatic), 2980 (CH_2 aliphatic), 1683, 1653 (3C=O), 1591 (C=N), 1508 (C=C), 538 (C–I). ^{1}H-NMR 400 MHz ($CDCl_3$, D_2O, ppm): δ 1.30 (t, 3H, J = 7.08 Hz, CH_3CH_2), 2.69 (s, 4H, piperazinyl-H), 3.32 (s, 2H, CH_2), 3.62 (s, 4H, piperazinyl-H), 4.18 (q, 2H, J = 7.08 Hz, CH_3CH_2), 7.60 (d, 1H, J = 8.72 Hz, phenyl-H), 7.84 (d, 2H, J = 5.36 Hz, pyridyl-H), 8.05 (s, 1H, phenyl-H), 8.53 (d, 1H, J = 8.72 Hz, phenyl-H), 8.85 (d, 2H, J = 5.36 Hz, pyridyl-H), 9.38 (s, 1H, NH exchanged by D_2O), 10.53 (s, 1H, NH exchanged by D_2O), 12.14 (s, 1H, NH exchanged by D_2O). Anal. calcd for $C_{22}H_{25}IN_6O_5$ (580.38): C, 45.53; H, 4.34; N, 14.48. Found: C, 45.70; H, 4.19; N, 14.50.

*N-(4-Iodo-2-{[2-(4-phenylpiperazin-1-ylacetyl)hydrazinyl]carbonyl} phenyl)pyridine-3-carboxamide (**12h**).* Mp: 240–242 °C. Yield: 68%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3277 (NHs), 3103 (CH aromatic), 2891 (CH_2 aliphatic), 1695, 1678, 1647 (2C=O), 1593 (C=N), 1556–1452 (C=C), 511 (C–I). ^{1}H-NMR 400 MHz ($CDCl_3$-d_6, D_2O, ppm): δ 2.88 (t, 4H, J = 4.56 Hz, piperazinyl-H), 3.33 (t, 4H, J = 4.6 Hz, piperazinyl-H), 3.36 (s, 2H, CH_2), 6.93 (t, 1H, J = 7.4 Hz, phenyl-H), 6.97 (d, 2H, J = 8.32 Hz, phenyl-H), 7.32 (t, 2H, J = 8.32 Hz, phenyl-H), 7.77 (dd, 1H, J = 1.80, 8.88 Hz, iodophenyl-H), 7.84 (dd, 2H, J = 1.48, 4.48 Hz, pyridyl-H), 8.04 (d, 1H, J = 1.72 Hz, iodophenyl-H), 8.61 (d, 1H, J = 8.88 Hz, iodophenyl-H), 8.83 (d, 2H, J = 6.00 Hz, pyridyl-H), 9.58 (s, 2H, NH exchanged by D_2O), 11.99 (s, 1H, NH exchanged by D_2O). Anal. calcd for $C_{25}H_{25}IN_6O_3$ (584.41): C, 51.38; H, 4.31; N, 14.38. Found: C, 51.46; N, 4.16; N, 14.46.

3-Hydroxy-6-iodo-2-(pyridin-3-yl)quinazolin-4(3*H*)-one (13a). A mixture of (pyridin-3-yl)-benzoxazin-4-one **6a** (3.5 g, 10 mmol) and hydroxylamine hydrochloride (0.69 g, 10 mmol) in dry pyridine (10 mL) was heated under reflux for 8 h. The product obtained upon cooling was filtered off, washed with diluted acetic acid and recrystallized from ethanol. Mp: 317–319 °C. Yield: 48%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3151 (OH), 3082 (CH aromatic), 1666 (C=O), 1608 (C=N), 1517 (C=C), 530 (C–I). ^{1}H-NMR 300 MHz (DMSO-d_6, D_2O, ppm): δ 7.64 (t, J = 5.10 Hz, 1H, pyridyl-H), 7.99 (dd, J = 1.80, 8.70 Hz, 1H, quinazolinyl-H), 8.26–8.29 (m, 2H, pyridyl-H), 8.40 (d, J = 8.7 Hz, 1H, quinazolinyl-H), 8.83 (s, 1H, quinazolinyl-H), 9.12 (s, 1H, pyridyl-H), 12.04 (s, 1H, OH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 365 (M^+, 0.15%), 350 (100%). Anal. calcd for $C_{13}H_8IN_3O_2$ (365.13): C, 42.76; H, 2.21; N, 11.51. Found: C, 42.90; H, 2.29; N, 11.66.

3-Hydroxy-6-iodo-2-(pyridin-4-yl)quinazolin-4(3*H*)-one (13b). Compound **13b** was similarly prepared and purified as compound **13a** but using (pyridin-4-yl)-benzoxazin-4-one (**6b**) as a starting material. Mp: 297–299 °C. Yield: 42%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3431 (OH), 3068 (CH aromatic), 1681 (C=O), 1593 (C=N), 1496 (C=C), 534 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 7.82 (d, 2H, J = 5.32 Hz, pyridyl-H), 8.01 (d, 1H, J = 8.64 Hz, quinazolinyl-H), 8.30 (d, 1H, J = 1.20 Hz, quinazolinyl-

H), 8.42 (d, 1H, J = 8.80 Hz, quinazolinyl-H), 8.85 (d, 2H, J = 5.32 Hz, pyridyl-H), 12.19 (s, 1H, OH exchanged by D_2O). Mass (m/z, rel. abundance): 365 (M^+, 9.23%), 350 (100%). Anal. calcd for $C_{13}H_8IN_3O_2$ (365.13): C, 42.76; H, 2.21; N, 11.51. Found: C, 42.87; H, 2.24; N, 11.67.

6-Iodo-4-oxo-2-(pyridin-3-yl)quinazoline-3(4*H*)-carboxamide (14a). To a suspension of the benzoxazinone **6a** (3.5 g, 10 mmol) in glacial acetic acid (10 mL), anhydrous sodium acetate (1.64 g, 20 mmol) and urea (0.6 g, 10 mmol) were added. The mixture was heated under reflux for 2 h, cooled to room temperature and poured on ice/water. The separated solid was filtered, washed with water and recrystallized from methanol. Mp: 307–309 °C. Yield: 71%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3153, 3107 (NH_2), 3080 (CH aromatic), 1660 (2C=O), 1606 (C=N), 1516 (C=C), 530 (C–I). ^{1}H-NMR 300 MHz (DMSO-d_6, D_2O, ppm): δ 7.63 (dd, 1H, J = 4.8, 8.1 Hz, pyridyl-H), 7.99 (dd, 1H, J = 2.10, 8.10 Hz, quinazolinyl-H), 8.26–8.29 (m, 2H, pyridyl-H, quinazolinyl-H), 8.42 (d, 1H, J = 8.7 Hz, quinazolinyl-H), 8.81 (d, 1H, J = 4.8 Hz, pyridyl-H), 9.11 (s, 1H, pyridyl-H), 12.08 (s, 2H, NH_2 exchanged by D_2O). Mass (m/z, rel. abundance): 394 (M^+ + 2, 85%), 393 (M^+ + 1, 1.68%), 392 (M^+, 93.42%), 76 (100%). Anal. calcd for $C_{14}H_9IN_4O_2$ (392.15): C, 42.88; H, 2.31; N, 14.29. Found: C, 43.01; H, 2.28; N, 14.54.

6-Iodo-4-oxo-2-(pyridin-4-yl)quinazoline-3(4*H*)-carboxamide (14b). Compound **14b** was synthesized according to the adopted procedure for **14a** using (pyridin-4-yl)-benzoxazin-4-one (**6b**) and was recrystallized from methanol. Mp: 330–332 °C. Yield: 70%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3099, 3066 (NH_2), 3043 (CH aromatic), 1681 (2C=O), 1597 (C=N), 1496 (C=C), 534 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 7.83 (d, 2H, J = 5.80 Hz, pyridyl-H), 8.01 (dd, 1H, J = 1.72, 8.72 Hz, quinazolinyl-H), 8.30 (d, 1H, J = 1.88 Hz, quinazolinyl-H), 8.42 (d, 1H, J = 8.80 Hz, quinazolinyl-H), 8.76 (d, 2H, J = 5.80 Hz, pyridyl-H), 12.17 (s, 1H, NH_2 exchanged by D_2O). Mass (m/z, rel. abundance): 392 (M^+, 0.14%), 78 (100%). Anal. calcd for $C_{14}H_9IN_4O_2$ (392.15): C, 42.88; H, 2.31; N, 14.29. Found: C, 43.07; H, 2.32; N, 14.51.

6-Iodo-4-oxo-2-(pyridin-3-yl)quinazoline-3(4*H*)-carbothioamide (15a). A mixture of benzoxazinone **6a** (3.5 g, 10 mmol) and thiourea (0.76 g, 10 mmol) in glacial acetic acid (15 mL) containing sodium acetate anhydrous (1.64 g, 20 mmol), was refluxed for 4 h. Upon pouring on crushed ice/water, beige powder was obtained, filtered, washed with water and recrystallized from ethanol. Mp: 303–305 °C. Yield: 70%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3365 (NH_2), 1666 (C=O), 1606 (C=N), 1516 (C=C), 1159 (C=S), 530 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 7.54 (d, 1H, J = 8.56 Hz, quinazolinyl-H), 7.58 (dd, 1H, J = 4.84, 7.88 Hz, pyridyl-H), 8.12 (dd, 1H, J = 1.36, 8.48 Hz, quinazolinyl-H), 8.42–8.48 (m, 2H, quinazolinyl-H, pyridyl-H), 8.76 (d, 1H, J = 4.28 Hz, pyridyl-H), 9.28 (d, 1H, J = 1.44 Hz, pyridyl-H), 12.86 (s, 2H, NH_2 exchanged by D_2O). Mass (m/z, rel. abundance): 408 (M^+, 44.59%), 78 (100%). Anal. calcd for $C_{14}H_9IN_4OS$ (408.22): C, 41.19; H, 2.22; N, 13.72. Found: C, 41.30; H, 2.18; N, 13.85.

6-Iodo-4-oxo-2-(pyridin-4-yl)quinazoline-3(4*H*)-carbothioamide (15b). This compound was prepared and purified as per the previously mentioned procedure.

Mp: 326–328 °C. Yield: 66%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3400 (NH_2), 3043 (CH aromatic), 1680 (C=O), 1597 (C=N), 1496 (C=C), 1085 (C=S), 534 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 7.83 (d, 2H, J = 5.84 Hz, pyridyl-H), 8.00 (dd, 1H, J = 1.76, 8.76 Hz, quinazolinyl-H), 8.30 (d, 1H, J = 1.84 Hz, quinazolinyl-H), 8.42 (d, 1H, J = 8.80 Hz, quinazolinyl-H), 8.84 (d, 2H, J = 5.84 Hz, pyridyl-H), 12.24 (s, 2H, NH_2 exchanged by D_2O). Mass (m/z, rel. abundance): 408 (M^+, 0.97%), 350 (100%). Anal. calcd for $C_{14}H_9IN_4OS$ (408.22): C, 41.19; H, 2.22; N, 13.72. Found: C, 41.34; H, 2.24; N, 13.80.

6-Iodo-4-oxo-2-(pyridin-3-yl)quinazoline-3(4*H*)-carbothiohydrazide (16a). A solution of the benzoxazine derivative **6a** (3.5 g, 10 mmol) and thiosemicarbazide (0.75 g, 10 mmol) in glacial acetic acid (15 mL) in the presence of sodium acetate (1.64 g, 20 mmol) was refluxed for 6 h. The reaction was poured onto ice/water and the obtained product was filtered off, washed with water and recrystallized from ethanol to give white crystals. Mp: 230–232 °C. Yield: 71%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3290 (NH_2), 3176 (NH), 3088 (CH aromatic), 1685 (C=O), 1591 (C=N), 1498 (C=C), 540 (C–I). ^{1}H-NMR 300 MHz (DMSO-d_6, D_2O, ppm): δ 7.55 (d, 1H, J = 8.40 Hz, quinazolinyl-H), 8.05 (d, 1H, J = 7.80 Hz, pyridyl-H), 8.12–8.20 (m, 1H, pyridyl-H), 8.22 (d, 1H, J = 2.10 Hz, pyridyl-H), 8.46 (d, 1H, J = 4.00 Hz, quinazolinyl-H), 8.69 (dd, 1H, J = 4.8, 12.90 Hz, quinazolinyl-H), 8.85 (d, 1H, J = 20.40, pyridyl-H), 10.17 (s, 2H, NH_2 exchanged by D_2O), 10.43 (s, 1H, NH exchanged by D_2O). Mass (m/z, rel. abundance): 423 (M^+, 0.67%), 349 (100%). Anal. calcd. for $C_{14}H_{10}IN_5OS$ (423.23): C, 39.73; H, 2.38; N, 16.55. Found: C, 39.89; H, 2.41; N, 16.79.

6-Iodo-4-oxo-2-(pyridin-4-yl)quinazoline-3(4*H*)-carbothiohydrazide (16b). Compound **16b** was prepared by applying the same procedure used for preparation of **16a** and was recrystallized from ethanol to give beige crystals. Mp: 210–212 °C. Yield: 60%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3290 (NH_2), 3242 (NH), 3062 (CH aromatic), 1681 (C=O), 1591 (C=N), 1504 (C=C), 540 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 7.55–7.60 (m, 1H, quinazolinyl-H), 7.70 (d, 2H, J = 5.12 Hz, pyridyl-H), 8.22 (d, 1H, J = 8.52 Hz, quinazolinyl-H), 8.48 (s, 1H, quinazolinyl-H), 8.75 (d, 2H, J = 5.12 Hz, pyridyl-H), 10.16 (s, 1H, NH_2 exchanged by D_2O), 10.43 (s, 1H, NH exchanged by D_2O). Mass (m/z, rel. abundance): 423 (M^+, 0.61%), 64 (100%). Anal. calcd. for $C_{14}H_{10}IN_5OS$ (423.23): C, 39.73; H, 2.38; N, 16.55. Found: C, 39.85; H, 2.37; N, 16.68.

6-Iodo-3-(4-un/substituted phenylamino)-2-(pyridin-3-yl)quinazolin-4(3*H*)-one (17a–c). To a suspension of benzoxazinone **6a** (3.5 g, 10 mmol) in absolute ethanol (20 mL), the required hydrazine derivative (10 mmol) was added and the reaction mixture was heated under reflux for 8–12 h. Then concentrated. Upon cooling the separated solid mass was filtered, washed with water and recrystallized from absolute ethanol to afford the titled derivatives **17a–c**.

*6-Iodo-3-(phenylamino)-2-(pyridin-3-yl)quinazolin-4(3H)-one (**17a**).* Mp: 176–178 °C. Yield: 55%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3325 (NH), 2922 (CH aromatic), 1681 (C=O), 1645 (C=N), 1598 (C=C), 520 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 6.74 (t, 1H, J = 7.28 Hz, phenyl-H), 6.84 (d, 2H, J = 7.72 Hz, phenyl-H), 7.16 (t, 2H, J = 7.72 Hz, phenyl-H), 7.59 (dd, 1H, J = 4.84, 7.92 Hz, pyridyl-H), 7.98 (d, 1H, J = 8.80 Hz, quinazolinyl-H), 8.20–8.23 (m, 1H, pyridyl-H),

8.30–8.32 (m, 2H, quinazolinyl-H), 8.78 (dd, 1H, J = 1.16, 4.68 Hz, pyridyl-H), 9.05 (d, 1H J = 1.76 Hz, pyridyl-H), 12.03 (s, 1H, NH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 440 (M^+, 100%). Anal. calcd for $C_{19}H_{13}IN_4O$ (440.24): C, 51.84; H, 2.98; N, 12.73. Found: C, 51.99; H, 3.03; N, 12.94.

*3-[(4-Fluorophenyl)amino]-6-iodo-2-(pyridin-3-yl)quinazolin-4(3H)-one (**17b**).* Mp: 310–312 °C. Yield: 52%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3325 (NH), 2924 (CH aromatic), 1681 (C=O), 1645 (C=N), 1598 (C=C), 501 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 7.00 (dd, 2H, J = 4.52, 8.88 Hz, phenyl-H), 7.17 (t, 2H, J = 4.72, 7.76 Hz, phenyl-H), 7.65 (dd, 1H, J = 4.72, 7.76 Hz, pyridyl-H), 8.01 (dd, 1H, J = 1.88, 8.68 Hz, quinazolinyl-H), 8.27–8.30 (m, 2H, pyridyl-H, quinazolinyl-H), 8.42 (d, J = 8.76 Hz, 1H, quinazolinyl-H), 8.82 (d, J = 3.80 Hz, 1H, pyridyl-H), 9.11 (s, 1H, pyridyl), 12.06 (s, 1H, NH exchanged by D_2O). ^{13}C-NMR (100 MHz DMSO-d_6, ppm): δ 79.46, 87.38, 116.10, 116.33, 117.07, 117.15, 120.06, 123.14, 124.72, 135.95, 139.56, 140.22, 142.95, 148.18, 152.71, 162.21 (C–F), 168.70 (C=O). Mass (*m*/*z*, rel. abundance): 458 (M^+, 8.24%), 78 (100%). Anal. calcd for $C_{19}H_{12}FIN_4O$ (458.23): C, 49.80; H, 2.64; N, 12.23. Found: C, 49.95; H, 2.68; N, 12.40.

*3-[(4-Chlorophenyl)amino]-6-iodo-2-(pyridin-3-yl)quinazolin-4(3H)-one (**17c**).* Mp: 254–256 °C. Yield: 65%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3265 (NH), 3082 (CH aromatic), 1681 (C=O), 1583 (C=N), 1487 (C=C), 520 (C–I). ^{1}H-NMR 300 MHz (DMSO-d_6, D_2O, ppm): δ 6.67 (d, 2H, J = 8.70 Hz, phenyl-H), 7.15 (d, 2H, J = 8.70 Hz, phenyl-H), 7.47 (d, 1H, J = 6.78 Hz, pyridyl-H), 7.68 (d, 1H, J = 8.40 Hz, quinazolinyl-H), 8.11 (d, 1H, J = 7.80 Hz, pyridyl-H), 8.22 (d, 1H, J = 8.70 Hz, quinazolinyl-H), 8.24 (s, 1H, quinazolinyl-H), 8.65 (s, 1H, pyridyl-H), 8.92 (s, 1H, pyridyl-H), 9.30 (s, 1H, NH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 474 (M^+, 49.72%), 476 (M^+ + 2, 17.35%), 349 (100%). Anal. calcd for $C_{19}H_{12}ClIN_4O$ (474.68): C, 48.07; H, 2.55; N, 11.80. Found: C, 48.28; H, 2.61; N, 11.94.

6-Iodo-3-(4-un/substituted phenylamino)-2-(pyridin-4-yl)quinazolin-4(3*H*)-one (17d–f). The quinazolinones **17d–f** were prepared and purified according to the previously mentioned procedure starting from the benzoxazinone **6b**.

*6-Iodo-3-(phenylamino)-2-(pyridin-4-yl)quinazolin-4(3H)-one (**17d**).* Mp: 239–241 °C. Yield: 40%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3304 (NH), 3057 (CH aromatic), 1672 (C=O), 1647 (C=N), 1519 (C=C), 534 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, D_2O, ppm): δ 6.74 (t, 1H, J = 7.28 Hz, phenyl-H), 6.84 (d, 2H, J = 7.88 Hz, phenyl-H), 7.16 (t, 2H, J = 7.64 Hz, phenyl-H), 7.77 (d, 2H, J = 5.68 Hz, pyridyl-H), 7.99 (s, 1H, quinazolinyl-H), 8.31–8.33 (m, 2H, quinazolinyl-H), 8.80 (d, 2H, J = 5.68 Hz, pyridyl-H), 10.77 (s, 1H, NH exchanged by D_2O). Anal. calcd for $C_{19}H_{13}IN_4O$ (440.24): C, 51.84; H, 2.98; N, 12.73. Found: C, 51.97; H, 2.96; N, 12.91.

*3-[(4-Fluorophenyl)amino]-6-iodo-2-(pyridin-4-yl)quinazolin-4(3H)-one (**17e**).* Mp: 234–236 °C. Yield: 42%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3240 (NH), 2922 (CH aromatic), 1678 (C=O), 1645 (C=N), 1598 (C=C), 1230 (C–F), 501 (C–I). ^{1}H-NMR 400 MHz (DMSO-d_6, ppm): δ 6.05 (d, 1H, J = 8.76 Hz, quinazolinyl-H), 7.09 (t, 2H, J = 8.80 Hz, phenyl-H), 7.30 (dd, 2H, J = 4.72, 8.84 Hz, phenyl-H), 7.52 (d, 2H, J = 5.92 Hz, pyridyl-H), 7.56 (d, 1H, J = 1.72 Hz, quinazolinyl-H), 8.19 (d, 1H, J = 1.76 Hz, quinazolinyl-H), 8.52 (d, 2H, J = 5.92 Hz, pyridyl-H), 10.06 (s, 1H, NH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 458 (M^+, 30.88%), 110 (100%). Anal. calcd for $C_{19}H_{12}FIN_4O$ (458.23): C, 49.80; H, 2.64; N, 12.23. Found: C, 49.98; H, 2.67; N, 12.39.

*3-[(4-Chlorophenyl)amino]-6-iodo-2-(pyridin-4-yl)quinazolin-4(3H)-one (**17f**).* Mp: 259–261. Yield: 51%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3290 (NH), 3180 (CH aromatic), 1656 (C=O), 1643 (C=N), 1483 (C=C), 755 (C–Cl), 520 (C–I). ^{1}H-NMR 300 MHz (DMSO-d_6, ppm): δ 7.35 (d, 2H, J = 7.80 Hz, phenyl-H), 7.45 (d, 2H, J = 7.80 Hz, phenyl-H), 7.55 (d, 1H, J = 8.70 Hz, quinazolinyl-H), 8.01 (d, 2H, J = 6.00 Hz, pyridyl-H), 8.20 (s, 1H, quinazolinyl-H), 8.69 (d, 2H, J = 6.00 Hz, pyridyl-H), 9.27 (s, 1H, quinazolinyl-H), 10.84 (s, 1H, NH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 474 (M^+, 35.35%), 476 (M^+ + 2, 11.89%), 126 (100%). Anal. calcd for $C_{19}H_{12}ClIN_4O$ (474.68): C, 48.07; H, 2.55; N, 11.80. Found: C, 48.31; H, 2.60; N, 11.94.

4-(6-Iodo-4-oxo-2-(pyridin-3-yl)quinazolin-3(4*H*)-yl)benzoic acid (18a). Equimolar amounts of benzoxazin-4-one **6a** (0.70 g, 2 mmol) and *p*-aminobenzoic acid (0.28 g, 2 mmol) were fused together at 200 °C in an oil bath for 1 h. The mixture was cooled, dissolved in boiling glacial acetic acid (30 mL) and filtered; the filtrate was concentrated up to (10 mL) *in vacuo* and cooled. The resulting solid was filtered, washed with water, dried and recrystallized from glacial acetic acid. Mp: 307–309 °C. Yield: 50%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3292 (OH), 1685 (2C=O), 1635 (C=N), 1508 (C=C), 540 (C–I). ^{1}H-NMR 300 MHz (DMSO-d_6, ppm): δ 7.58 (dd, 1H, J = 4.80, 7.80 Hz, pyridyl-H), 7.82 (d, 2H, J = 8.70 Hz, phenyl-H), 7.92 (d, 2H, J = 8.70 Hz, phenyl-H), 7.99 (d, 1H, J = 2.10 Hz, quinazolinyl-H), 8.20–8.23 (m, 1H, pyridyl-H), 8.29 (d, 1H, J = 1.80 Hz, quinazolinyl-H), 8.40 (s, 1H, quinazolinyl-H), 8.76 (d, 1H, J = 4.20 Hz, pyridyl-H), 9.04 (s, 1H, J = 1.76 Hz, pyridyl-H), 11.23 (s, 1H, OH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 469 (M^+, 1.01%), 78 (100%). Anal. calcd for $C_{20}H_{12}IN_3O_3$ (469.23): C, 51.19; H, 2.58; N, 8.96. Found: C, 51.34; H, 2.60; N, 9.08.

4-(6-Iodo-4-oxo-2-(pyridin-4-yl)quinazolin-3(4*H*)-yl)benzoic acid (18b). This compound was prepared and purified as per the previously mentioned procedure, using the benzoxazinone derivative **6b**. Mp: 244–246 °C. Yield: 55%. I.R. (KBr, cm^{-1}): $\tilde{\nu}_{max}$ 3334 (OH), 1681 (2C=O), 1589 (C=N), 1506 (C=C), 519 (C–I). ^{1}H-NMR 300 MHz (DMSO-d_6, ppm): δ 7.35 (d, 2H, J = 7.80 Hz, phenyl-H), 7.45 (d, 2H, J = 7.80 Hz, phenyl-H), 7.55 (d, 1H, J = 8.70 Hz, quinazolinyl-H), 8.01 (d, 2H, J = 6.00 Hz, pyridyl-H), 8.20 (d, 1H, J = 8.64 Hz, quinazolinyl-H), 8.69 (d, 2H, J = 6.00 Hz, pyridyl-H), 9.27 (s, 1H, quinazolinyl-H), 12.12 (s, 1H, OH exchanged by D_2O). Mass (*m*/*z*, rel. abundance): 469 (M^+, 42.74%), 78 (100%). Anal. calcd for $C_{20}H_{12}IN_3O_3$ (469.23): C, 51.19; H, 2.58; N, 8.96. Found: C, 51.31; H, 2.62; N, 9.03.

(2) Biology

(a) *In vitro* cytotoxicity. The *in vitro* cytotoxicity of the newly synthesized compounds against a panel of nine cancer cell lines and one non-tumorigenic cell line was assessed using the MTT assay according to Mosmann's method.[41] The MTT assay which was developed in 1983, is based on the reduction of the soluble 3-(4,5-methyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) into a blue-purple formazan product, mainly by mitochondrial reductase activity inside living cells. Mosmann

showed that MTT assay has very wide applicability for measuring survival and/or proliferation of various cells and can potentially be applied to any assay in which living cells must be distinguished from dead cells or a lack of cells. The assay also has potential value for quantitative and rapid measurement of cell death.

The cells used in cytotoxicity assay were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum. Cells suspended in the medium (2×10^4/mL) were plated in 96-well culture plates and incubated at 37 °C in a 5% CO_2 incubator. After 12 h, the test sample (2 μL) was added to the cells in 96-well plates and cultured at 37 °C for 3 days.

The cultured cells were mixed with 20 μL of MTT solution and incubated for 4 h. at 37 °C. The supernatant was carefully removed from each well and 100 μL of DMSO were added to each well to dissolve the formazan crystals which were formed by the cellular reduction of MTT. After mixing with a mechanical plate mixer, the absorbance of each well was measured by a microplate reader using a test wavelength of 570 nm.

(b) *In vivo* cytotoxicity. Female athymic pathogen-free nude mice (nu/nu, 4–6 weeks) were purchased and maintained in accordance with the guidelines of the so adopted Guide for the Care and Use of Laboratory Animals 8th Edition 2011, and as approved by the Research Ethics Committee (REC) of Faculty of Pharmacy, Cairo University. To establish MCF-7 human breast cancer xenografts, each of the female nude mice was first implanted with a 60 day subcutaneously (s.c.) slow release estrogen pellet (SE-121, 1.7 mg 17β-estradiol/pellet). The next day, cultured MCF-7 cells were harvested from confluent monolayer cultures, washed twice with serum-free medium, resuspended and injected s.c. (5×10^6 cells, total volume 0.2 mL) into the left inguinal area of the mice. All animals were monitored for activity, physical condition, body weight, and tumor growth. Tumor size was determined by caliper measurement in two perpendicular diameters of the implant every other day. Tumor volume was calculated by the formula, $1/2a \times b^2$ where "a" is the long diameter and "b" is the short diameter (in cm).

The animals bearing human cancer xenografts were randomly divided into various treatment groups (of 5 animals each) and a control group (7–10 mice/group). The untreated control group received the vehicle only. For the MCF-7 xenograft model, tested compounds and the reference control drug sorafenib were dissolved in PEG400 : ethanol : saline (57.1 : 14.3 : 28.6, v/v/v), and were administered by intraperitoneal (i.p.) injection at doses equivalent of 5 or 10 mg kg^{-1}, every 48 h for 3 weeks were dosing and dose regimen were selected based on previous reports of sorafenib.[42]

(c) Kinase inhibitory assay. In a final reaction volume of 25 μL, kinase (5–10 mU) was incubated with 25 mM Tris pH 7.5, 0.02 mM EGTA, 0.66 mg mL^{-1} myelin basic protein, 10 mM magnesium acetate and [γ^{33}P-ATP] (specific activity approximately 500 cpm $pmol^{-1}$, concentration as required). The reaction was initiated by the addition of the Mg–ATP mixture. After incubation for 40 minutes at room temperature, the reaction was stopped by the addition of 5 μL of a 3% phosphoric acid solution. 10 μL of the reaction was then spotted onto a P30 ltermat and washed three times for 5 minutes in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

(3) Molecular docking

The molecular modeling study was carried out using Molecular Operating Environment (MOE 2009.10) software provided by chemical computing group, Canada. The energy minimization for the compounds was performed with MOE with MMFF94X force field and the partial charges were automatically calculated.[43,44]

Crystallographic complexes of ABL with its respective inhibitors imatinib and bosutinib (PDB IDs: 2HYY and 3UE4) were downloaded from the protein data bank available at http://www.rcsb.org/pdb. The protein-ligand complex obtained from the protein data bank was prepared for docking as follows: the enzyme was 3D protonated, where hydrogen atoms were added at their standard geometry, the partial charges were computed and the system was optimized.

Deletion of water of crystallization together with extra chains of the protein was then done. Finally, isolation of the active site and recognition of the involved amino acids was carried out then the backbone was hidden.

Docking was performed using Triangle Matcher placement method, poses were prioritized based on London dG scoring and refinement of the results was done using forcefield. The most stable docking model was selected per the best scored conformation predicted by the MOE scoring function.

Conflict of interest

The authors declare no conflict of interest.

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