Synthesis and Docking Studies of Some 1,2,3-Benzotriazine-4-one Derivatives as Potential Anticancer Agents

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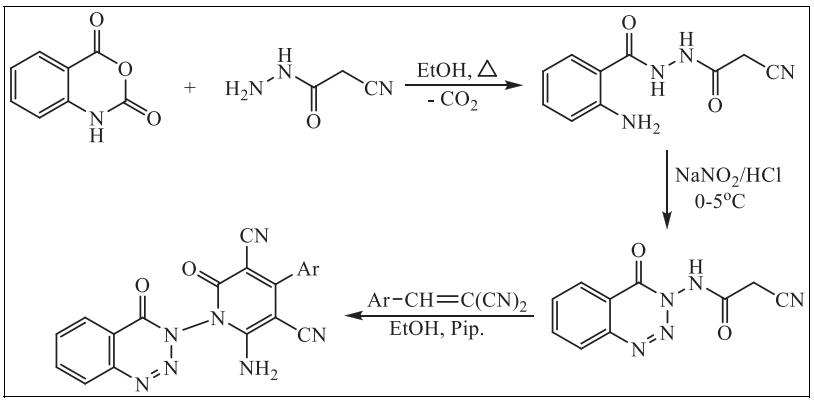


Newly synthesized 1,2,3-benzotriazine-4-one derivatives substituted at position-3 were characterized by various analytical and spectral techniques. The *in vitro* antitumor activity was evaluated against three different cell lines (liver cells cancer, colorectal cancer, and breast cancer), where compounds **7b**, **15**, and **25** showed strong antitumor activity with IC_{50} ranging from 5.54 to 16.26 μM. In addition, molecular modeling studies using MOE were performed to investigate their binding modes to the C-Met kinase active site. Docking results demonstrated that all new compounds recognized the active sites of C-Met kinase and form different types of bonding interactions with key active site amino acid residues.

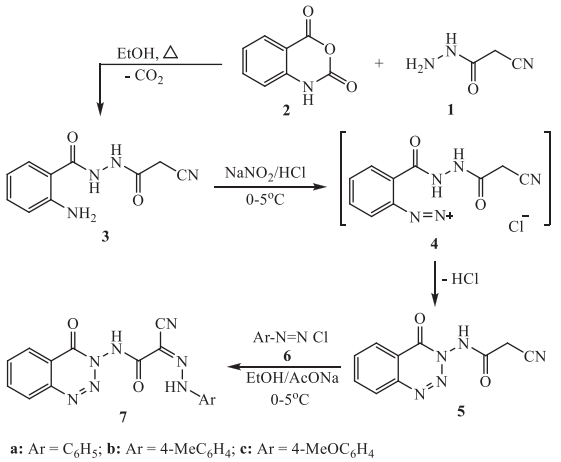
INTRODUCTION

The three possible isomers of triazine (1,2,3-triazines, 1,2,4-triazines, and 1,3,5-triazines) have great interest in medicinal chemistry due to their wide range of activities such as antimicrobial [1,2], anti-inflammatory [3,4], analgesic [5], antihistaminic [6], anticancer [7], antiviral [8], and antiangiogenic [9]. The benzo-fused derivatives of 1,2,3-triazines have attracted much attention in both the medicinal and agrochemical fields [10–12]. Many pharmacological properties for 1,2,3-benzotriazine-4-ones have been reported, including drugs with sedative, anesthetic, antitumor, antiarthritic, diuretic, and antitubercular activities [13–18]. In this paper, the key 2-cyano-*N*-(4-oxobenzo [*d*][1,2,3]triazin-3(4*H*)-yl) acetamide (**5**) has been synthesized and utilized as a precursor for various 1,2,3-benzotriazine-4-ones containing various heterocycles (e.g., pyridine, chromene, thiazole, and pyrazole) at position-3. The resulting series of 3-substituted 1,2,3-benzotriazine-4-ones was evaluated for their anticancer activities against liver cells cancer, colorectal cancer, and breast cancer.

RESULTS AND DISCUSSION

Chemistry. The reaction of cyanoacetic hydrazide (**1**) with isatoic anhydride (**2**) has been achieved by refluxing in ethanol to furnish 2-amino-*N*′-(2-cyanoacetyl) benzohydrazide (**3**) in 67% yield. The reaction mechanism involves nucleophilic attack of the amino part from cyanoacetic hydrazide on the carbonyl group of isatoic anhydride, which leads to ring opening and decarboxylation to produce our target benzohydrazide **3** (Scheme 1). The chemical structure of this benzohydrazide was proved based on both elemental and spectral data. The IR absorption bands clearly indicated the presence of NH_2 and NH functions at 3459, 3363, and 3250 cm^{-1}, nitrile function (C ≡ N) at 2261 cm^{-1}, and two carbonyl functions at 1687 and 1654 cm^{-1}. The ^1H NMR spectrum revealed singlet for two protons at 3.78 ppm (CH_2), singlet at 6.40 ppm for two protons (NH_2), two triplet signals at 6.50 and 7.21, and two doublet signals at 6.74 and 7.53 ppm (aromatic protons). Finally, singlet at 10.16 ppm for the two protons of imine functions (NH).

Scheme 1. Synthetic pathway to 2-cyano-*N*-(4-oxobenzotriazin-3-yl) acetamides **5** and **7**.

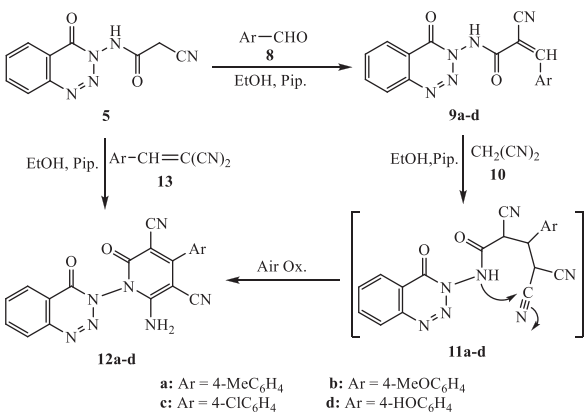


a: Ar = C_6H_5; **b:** Ar = 4-MeC_6H_4; **c:** Ar = 4-$MeOC_6H_4$

When 2-amino-*N*′-(2-cyanoacetyl) benzohydrazide (**3**) treated with nitrous acid ($NaNO_2$/HCl) at 0–5°C, it readily diazotized to form the intermediate diazonium chloride **4**, which underwent *in situ* intramolecular cyclization to afford 2-cyano-*N*-(4-oxobenzo [*d*][1,2,3]triazin-3(4*H*)-yl) acetamide (**5**) in 77% yield. The chemical structure of **5** was verified on the basis of spectral data, the IR exhibited absorptions at 3174 cm^{-1} corresponding to NH group, 2260 cm^{-1} for the nitrile function (C ≡ N) while absorptions at 1723 and 1693 cm^{-1} was attributed to the carbonyl functions (C=O). The protons of methylene group (CH_2) appeared as singlet at 4.17 ppm in the ^{1}H NMR spectrum. The methylene group of cyanoacetamide derivative **5** proved to be reactive toward electrophilic coupling reaction with aryl diazonium chlorides. Thus, it coupled with various diazotized anilines **6** (namely, aniline, 4-toluidine, and 4-anisidine) in ethyl alcohol containing sodium acetate at 0–5°C to produce the corresponding 2-oxo-2-((4-oxobenzotriazin-3-yl)amino)-*N*-aryl-acetohydrazonoyl cyanide derivatives **7a–c**. The structures of these synthesized hydrazones were characterized by spectral data. For example, the IR spectrum of **7b** showed absorption bands at 3327 and 3230 cm^{-1} referring to imino groups (NH), and nitrile group (C ≡ N) was detected at 2214 cm^{-1} in addition to two peaks that appeared at 1704 and 1682 cm^{-1} for the carbonyl groups (C=O). The ^{1}H NMR spectrum of **7c** revealed singlet at 3.77 ppm for three protons of methoxy group (OCH_3). The signals of aromatic protons resonated as two doublet at 6.99 and 7.72 ppm, two triplet at 8.03 and 8.20 ppm, and multiplet at 8.28–8.36 ppm. The protons of two NH groups were characterized as two singlet signals at 12.06 and 12.33 ppm.

Knoevenagel condensation of cyanoacetamide **5** with various para substituted benzaldehydes (namely, *p*-tolualdehyde, *p*-anisaldehyde, *p*-chlorobenzaldehyde, and *p*-hydroxybenzaldehyde) furnished the corresponding α,β-unsaturated nitrile derivatives **9a–d** (Scheme 2). The IR spectrum of **9a** was characterized by the presence of absorptions at 3287, 2123, and 1682 cm^{-1} corresponding to NH, nitrile, and carbonyl functions, respectively. The ^{1}H NMR of **9b** displayed singlet at 3.87 ppm for three protons (OCH_3), doublet at 7.14 ppm, and multiplet in the region 7.95–8.35 for the aromatic protons in addition to singlet at 9.16 ppm for the olefinic proton (CH=C). The reaction of α,β-unsaturated nitrile derivatives **9a–d** with malononitrile (**10**) has been achieved by heating the two reactants under reflux in ethyl alcohol and drops of piperidine to produce the corresponding *N*-benzotriazinyl-pyridone derivatives **12a–d**. The reaction proceeds *via* a nucleophilic attack of active methylene (from malononitrile) on the synthesized α,β-unsaturated nitrile **9** to form the Michael adduct **11** which underwent intramolecular cyclization and auto-oxidation achieve the desired product **12**. The chemical structures of **12a–d** have been secured by their IR, ^{1}H NMR, and their alternative synthesis from the reaction of the cyanoacetamide **5** with arylidene-malononitriles **13** (namely, 4-methylbenzylidene-malononitrile, 4-methoxybenzylidene-malononitrile, 4-chlorobenzylidene-malononitrile, and 4-hydroxybenzylidene-malononitrile) in boiling ethyl alcohol and few drops of piperidine. The spectroscopic data of the obtained *N*-benzotriazinyl-pyridones **12a–d** were in complete agreement with the assigned structures. IR spectrum of **12a** showed absorptions at 3424 and 3365 cm^{-1} for the amino group (NH_2), 2205 cm^{-1} for the nitrile function (C ≡ N) in addition to 1676 and 1625 cm^{-1} for carbonyl groups (C=O). ^{1}H NMR spectrum of the same compound exhibited singlet at 3.82 pm corresponding to three protons of (OCH_3) function, singlet at 7.22 ppm for the protons of amino group (NH_2) in addition to doublet, triplet and multiplet signals in the region 6.94–8.28 for the aromatic protons.

Scheme 2. Synthesis of *N*-benzotriazinyl-pyridones **12a–d**.

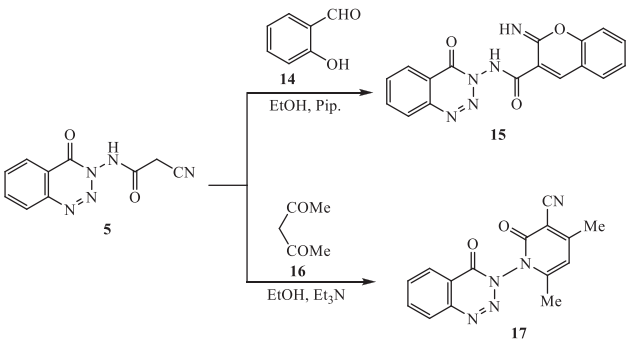


a: Ar = 4-MeC_6H_4 **b:** Ar = 4-$MeOC_6H_4$
c: Ar = 4-ClC_6H_4 **d:** Ar = 4-HOC_6H_4

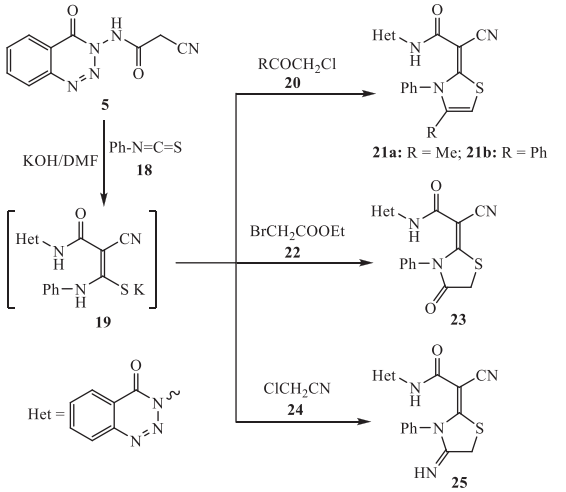
When cyanoacetamide **5** heated with salicylaldehyde (**14**) in ethanol and drops of piperidine for approximate time, 2-imino-*N*-(4-oxobenzo [*d*][1,2,3]triazin-3(4*H*)-yl)-2*H*-chromene-3-carboxamide (**15**) has been separated in moderate yield (Scheme 3). The infrared spectrum of **15** was characterized by absorptions at 3442, 1709, and 1650 cm^{-1} attributable to the NH, carbonyl (C=O) functions, respectively. The ^1H NMR spectrum revealed multiplet bands at 6.99–8.59 ppm assigned for the aromatic protons and singlet signal at 9.48 ppm corresponding to the proton of chromene C4. Furthermore, condensation of cyanoacetamide **5** with acetyl acetone (**16**) in refluxed ethanol and few drops of triethylamine furnished the corresponding *N*-benzotriazinyl-2-pyridinone scaffold **17**. In the ^1H NMR spectrum of **17**, the protons of two methyl groups ($2CH_3$) appeared as two singlet signals at 2.26 and 2.54 ppm and the proton at C-5 of the pyridine ring appeared as singlet signal at 6.25 ppm, while the aromatic protons appeared as two triplet signals at 7.49 and 8.11 ppm and two doublet signals at 8.29 and 8.43 ppm.

The basic catalyzed addition of benzotriazinyl cyanoacetamide **5** to phenyl isothiocyanate (**18**) was achieved by stirring the reactants in DMF containing solid potassium hydroxide for 6 h to yield the corresponding non-isolable potassium sulfide salt **19**, which underwent further *in situ* heterocyclization reactions with α-halogenated reagents **20**, **22**, and **24** to furnish the corresponding 3-phenylthiazole derivatives (Scheme 4). First, *in situ* treatment of **19** with chloroacetone and/or phenacyl chloride at room temperature prompted the formation of 2-cyano-2-(4-substituted-thiazol-2-ylidene)-*N*-(4-oxo-benzotriazin-3-yl) acetamides **21a** and **21b**, respectively. The IR spectrum of **21a** revealed absorptions at 3211 cm^{-1} referring to the NH function, 2194 cm^{-1} due to the nitrile function (C ≡ N), while the absorptions at 1712 and 1693 cm^{-1} were attributed to the carbonyl groups. The ^1H NMR spectrum of the same compound exhibited singlet signal at 2.50 ppm referring to three protons (CH_3), singlet at 5.20 ppm for the proton at C-5 of the thiazole ring,

Scheme 3. Synthesis of benzotriazine derivatives **15** and **17**.



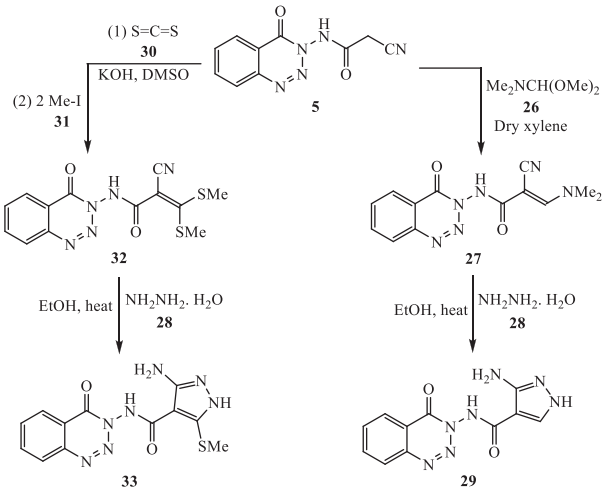
Scheme 4. Synthesis of 2-cyano-2-(thiazol-2-ylidene)-*N*-(4-oxo-benzotriazin-3-yl) acetamides **21**, **23**, and **25**.



multiplet in the region 7.04–8.38 ppm for the aromatic protons, and a further singlet signal deshielded at 10.87 ppm corresponding to one proton (NH). In addition, *in situ* addition of ethyl bromoacetate to **19** prompted the construction of 2-cyano-2-(4-oxo-thiazolidin-2-ylidene)-*N*-(4-oxobenzotriazin-3-yl) acetamide scaffold **23** with molecular formula $C_{19}H_{12}N_6O_3S$. The IR spectrum displayed the absorption of NH group at 3320 cm^{-1}, nitrile group (C ≡ N) at 2195 cm^{-1}, and three carbonyl groups at 1736, 1712, and 1677 cm^{-1}. Finally, *in situ* cyclization of **19** using chloroacetonitrile was responsible for the production of 2-cyano-2-(4-imino-thiazolidin-2-ylidene)-*N*-(4-oxobenzotriazin-3-yl) acetamide scaffold **25** in moderate yield. ^1H NMR spectrum was utilized to confirm the structure of **25**, the protons of methylene group (CH_2) appeared as singlet at 3.89 ppm, the aromatic protons resonated as multiplet at 7.32–8.31 ppm, and the protons of two NH groups appeared as two singlet signals at 10.24 and 11.13 ppm.

Enaminonitrile derivative **27** was easily synthesized through the reaction of cyanoacetamide **5** with dimethylformamide-dimethylacetal (**26**) by heating in dry xylene (Scheme 5). The chemical structure of **27** has been secured because of its spectroscopic analyses. The IR spectrum exhibited absorptions at 3342 cm^{-1} for the NH function, 2195 cm^{-1} for the nitrile function (C ≡ N), and 1688, 1668 cm^{-1} for the carbonyl groups (C=O). The ^1H NMR spectrum of **27** revealed two singlet signals at 3.25 and 3.36 ppm for the protons of two methyl groups N $(CH_3)_2$ and singlet signal at 7.87 ppm for the olefinic proton (CH=C). The aromatic protons resonated as two triplet and multiplet signals the region 7.98–8.30 ppm, while the proton of NH function was singlet at 11.15 ppm. The behavior of the enaminonitrile **27** toward hydrazine hydrate (an example of nitrogen nucleophiles)

Scheme 5. Synthesis of 5-amino-*N*-(4-oxobenzotriazin-3-yl)-1*H*-pyrazole-4-carboxamides **29** and **33**.



to attain polyfunctionally substituted azole was studied. Thus, 5-amino-*N*-(4-oxobenzotriazin-3-yl)-1*H*-pyrazole-4-carboxamide (**29**) was picked up as a sole product by boiling enaminonitrile **27** with hydrazine hydrate (**28**) in ethyl alcohol. The IR spectrum clearly indicated the lack of any absorption due to cyano function. In the ^{1}H NMR spectrum, the absence of singlet signals for the protons of methyl groups clearly indicated the loss of N $(CH_3)_2$ group. The reaction of cyanoacetamide **5** with carbon disulfide (**30**) in DMSO containing potassium hydroxide proceeded by stirring for 6 h at room temperature, followed by *in situ* addition of 2 mol of methyl iodide (**31**) gave the corresponding ketene dithioacetal derivative **32**. The presence of absorptions at 3245 cm^{-1} (NH), 2201 cm^{-1} (C ≡ N), and 1708 and 1683 cm^{-1} (C=O) in the IR spectrum secured the structure of **32**. The ^{1}N MR spectrum displayed two singlet signals at 2.66 and 2.73 ppm for the protons of two methyl groups, triplet and multiplet signals in the region 8.01–8.34 for the aromatic protons, in addition to singlet at 12.33 for the proton of NH group. The aminopyrazole derivative **33** was synthesized in 46% yield by boiling a suspension of ketene dithioacetal **32** with hydrazine hydrate (**28**) in ethyl alcohol for 4 h as shown in Scheme 5. The IR spectrum of **33** exhibited absorptions at 3443 and 3324 cm^{-1} for the (NH_2 and NH) function, in addition to broad absorption near 1620 cm^{-1} for the carbonyl function (C=O). The ^{1}H NMR spectrum displayed singlet at 2.61 ppm for three protons (CH_3), singlet at 7.33 ppm for two protons (NH_2), triplet and multiplet signals in the region 7.52–8.24 ppm for the aromatic protons, and two singlet signals at 10.51 and 11.87 ppm for the protons of two NH groups.

Anticancer activity (*in vitro* cytotoxicity activity). The pharmacological activities of the synthesized 1,2,3-benzotriazine-4-one scaffolds were performed against three types of human cancer cell lines: HepG2 (hepatocellular carcinoma), HTC-116 (colorectal carcinoma), and MCF-7 (mammary gland breast cancer). Cytotoxic potential of these 1,2,3-benzotriazine-4-ones on carcinogenic cells was tested by the MTT method [19,20]. The cytotoxicity evaluation involved the use of doxorubicin as antitumor drug reference standard. From the obtained results in Table 1, we observe that the tested 1,2,3-benzotriazine-4-one compounds displayed important differences in the cytotoxic activity. Thus, it can be mentioned that varying the heterocyclic moiety at position-3 of the benzotriazine-4-one nucleus causes significant changes in cytotoxicity. Among the tested compounds, compounds **7b** (bearing tolyl-hydrazonoyl cyanide group) and **25** (4-imino-thiazolidin-2-ylidene group) were found to be the most potent scaffolds against all three cell lines with IC_{50} values from 5.54 to 11.84 μM. Luckily, compounds **15** (having 2-oxo-2*H*-chromene moiety) and **21b** (4-phenyl-thiazol-2-ylidene moiety) displayed strong activities toward HCT-116 and MCF-7 and moderate activities toward HepG2. Moreover, compounds **7a**, **9b**, **12d**, **21a**, and **23** possessed moderate anti-proliferative activities against HepG2, HCT-116, and MCF-7 cell lines. Unfortunately, some of the synthesized 1,2,3-benzotriazine-4-one derivatives **5**, **7c**, **9c**, **17**, **27**,

Table 1

Cytotoxic activity of the synthesized 1,2,3-benzotriazine-4-one scaffolds against HepG2, HCT-116, and MCF-6 cancer cell lines.

Compound	*In vitro* Cytotoxicity IC_{50} (μM)		
	HepG2	HCT-116	MCF-7
DOX	**4.50 ± 0.2**	**5.23 ± 0.3**	**4.17 ± 0.2**
5	78.36 ± 3.9	55.62 ± 3.3	52.60 ± 3.5
7a	68.15 ± 3.7	30.64 ± 2.3	23.09 ± 1.9
7b	7.67 ± 0.9	6.59 ± 0.5	8.28 ± 0.7
7c	85.57 ± 4.0	79.34 ± 4.1	88.93 ± 4.8
9a	57.36 ± 2.2	61.20 ± 2.8	64.38 ± 3.1
9b	52.40 ± 2.8	35.89 ± 2.6	34.09 ± 2.9
9c	86.17 ± 4.2	67.71 ± 3.8	78.19 ± 3.9
9d	60.52 ± 2.8	58.22 ± 2.6	56.72 ± 2.4
12a	41.78 ± 1.4	38.24 ± 1.8	40.11 ± 1.7
12b	47.36 ± 1.6	45.38 ± 1.8	50.11 ± 2.3
12c	55.03 ± 1.9	49.58 ± 1.2	50.22 ± 1.6
12d	69.91 ± 3.7	46.69 ± 3.1	37.21 ± 3.0
15	45.54 ± 2.5	20.35 ± 1.7	15.39 ± 1.6
17	>100	75.37 ± 3.9	95.47 ± 5.1
21a	32.85 ± 2.1	27.80 ± 2.0	28.23 ± 2.4
21b	25.74 ± 1.8	17.36 ± 1.4	13.77 ± 1.2
23	16.26 ± 1.3	13.92 ± 1.2	9.15 ± 0.9
25	5.54 ± 0.6	10.53 ± 0.9	11.84 ± 1.1
27	58.75 ± 3.4	64.18 ± 3.8	53.15 ± 3.6
29	54.68 ± 1.6	58.33 ± 1.8	49.26 ± 1.4
32	93.62 ± 4.5	95.55 ± 4.7	>100
33	64.12 ± 3.8	68.24 ± 3.1	56.44 ± 2.2

IC_{50} values are the mean ± SD of three separate experiments. DOX, doxorubicin.

and **32** did not manifest significant anti-proliferative activities against the tested cell lines.

Molecular docking study. Motivated by the promising results of the antitumor screening data of our newly synthesized benzotriazine-4-one scaffolds and the grounds of having two FDA approved c-Met kinase inhibitors (Crizotinib and Cabozantinib) for cancer treatment, we performed molecular docking studies to investigate the underlying mechanism of possible molecular interaction of these compounds with c-Met tyrosine kinase and the receptor of hepatocyte growth factor/scatter factor (HGF/SF). Simulation of both possible binding modes and affinities of this series of new ligands was performed using MOE v10.2009 software and c-Met kinase active site amino acids (PDB. I.D.3ce3). The native cocrystallized ligand was redocked in the active site after preparation of the protein using standard procedure to initially validate docking process. The results of validation showed the program docked the ligand in the active site using the same binding pose as the X-ray crystal structure, as reflected thorough a low relative mean standard deviation (1.48 A˚) (Fig. 1). The

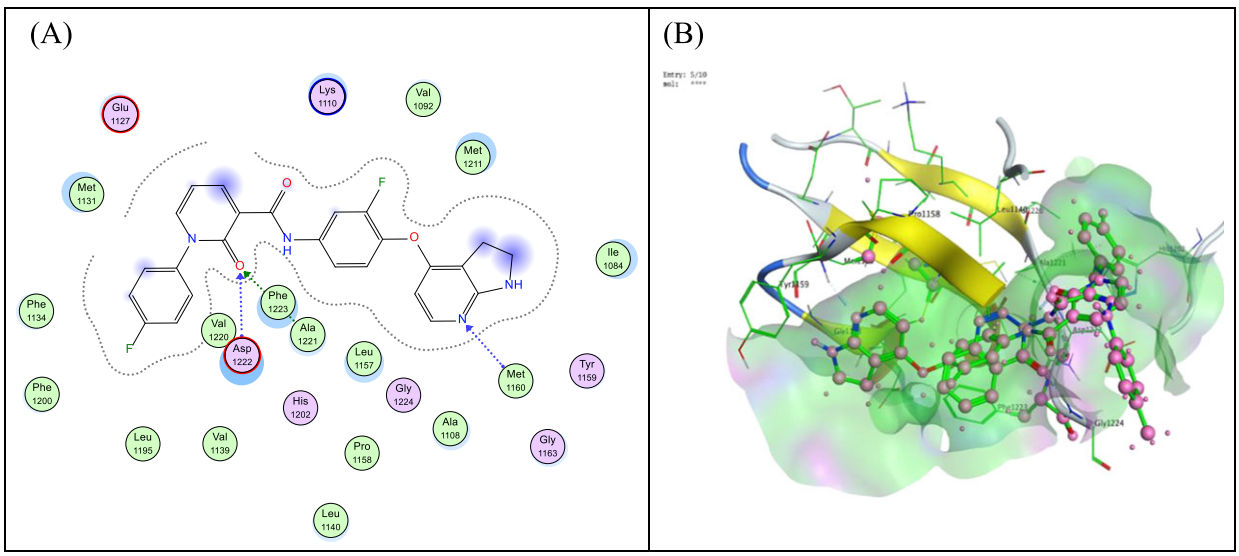


Figure 1. (A) Docking validations 2D ligand interaction and (B) 3D superposition of native ligand and redocked native ligand at the binding site of C-Met kinase [PDB ID: 3ce3; relative mean standard deviation (RMSD) = 1.4855; S = −8.2936 kcal/mol]. [Color figure can be viewed at wileyonlinelibrary.com]

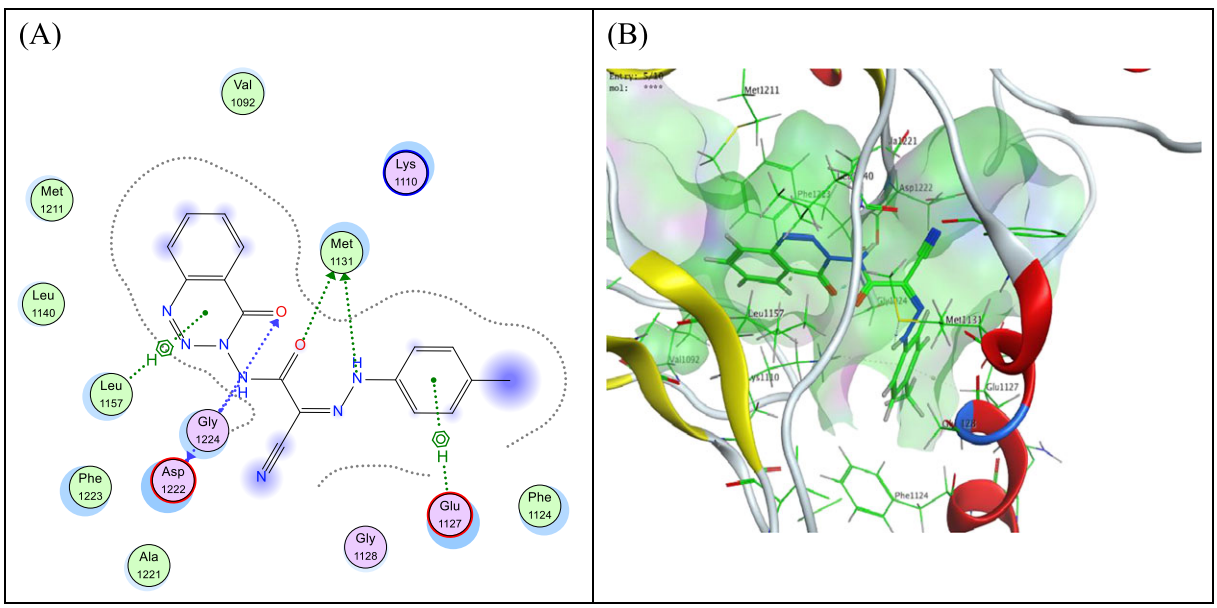


Figure 2. (A) 2D and (B) 3D ligand interaction of **7b** with the active sides amino acid of C-Met kinase (S = −5.9088) in comparison with native ligands. [Color figure can be viewed at wileyonlinelibrary.com]

native ligand was bound to the active site's amino acids with a network of hydrogen bonds, thus resulting in a binding energy score of S = −8.2936 kcal/mol (Fig. 1).

The benzotriazine-4-one scaffolds have been prepared for docking simulation as described in the experimental (docking studies); results show that all members of this series of compounds were able to recognize the active site of the c-Met kinase and perform different types of bonding interactions with the key active site amino acid residues. As representatives, 2D and 3D binding interactions of the candidates **7b**, **15**, and **25** are displayed in the top and bottom panels of Figures 2, 3, and 4, respectively. Benzotriazine-4-one compound **15** accommodated favorably in the active pocket of the receptor and made binding interactions with the key active site amino acid residues.

Compound **7b** formed four hydrogen bonds, one between the C=O of the triazine ring and GLY 1224 (3.0A˚) and the other between the C=O of the amidic group and MET1131 (3.18 A˚). The third bond is between the NH of the amidic group and ASP1222 (2.62 A˚), while the last hydrogen bond is between C=N—NH and

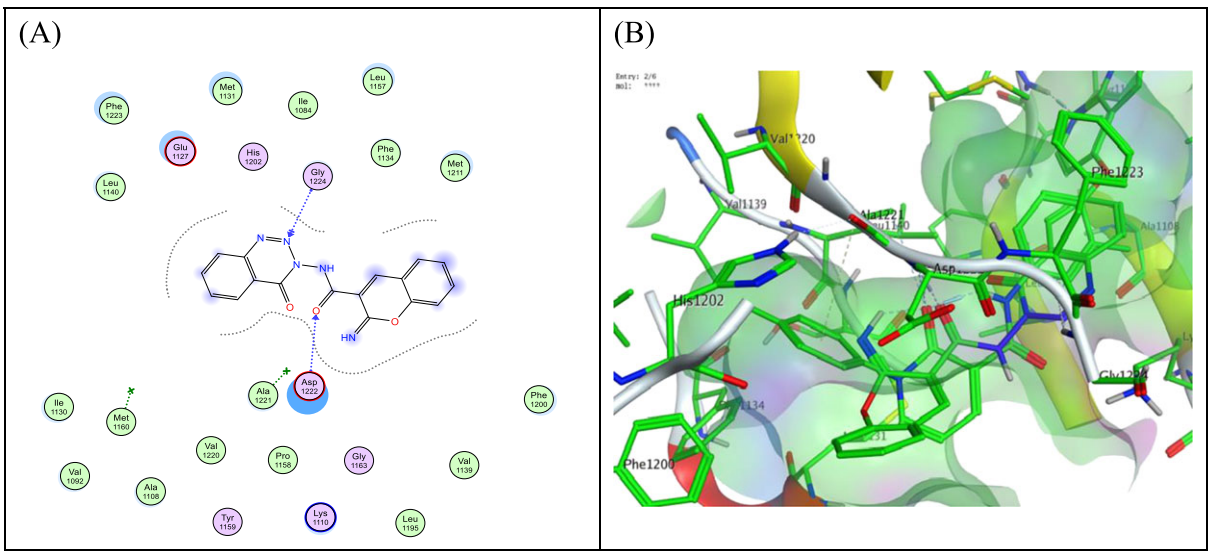


Figure 3. (A) 2D and (B) 3D ligand interaction of **15** with the active sides amino acid of C-Met kinase (S = −22.1657) in comparison with native ligands. [Color figure can be viewed at wileyonlinelibrary.com]

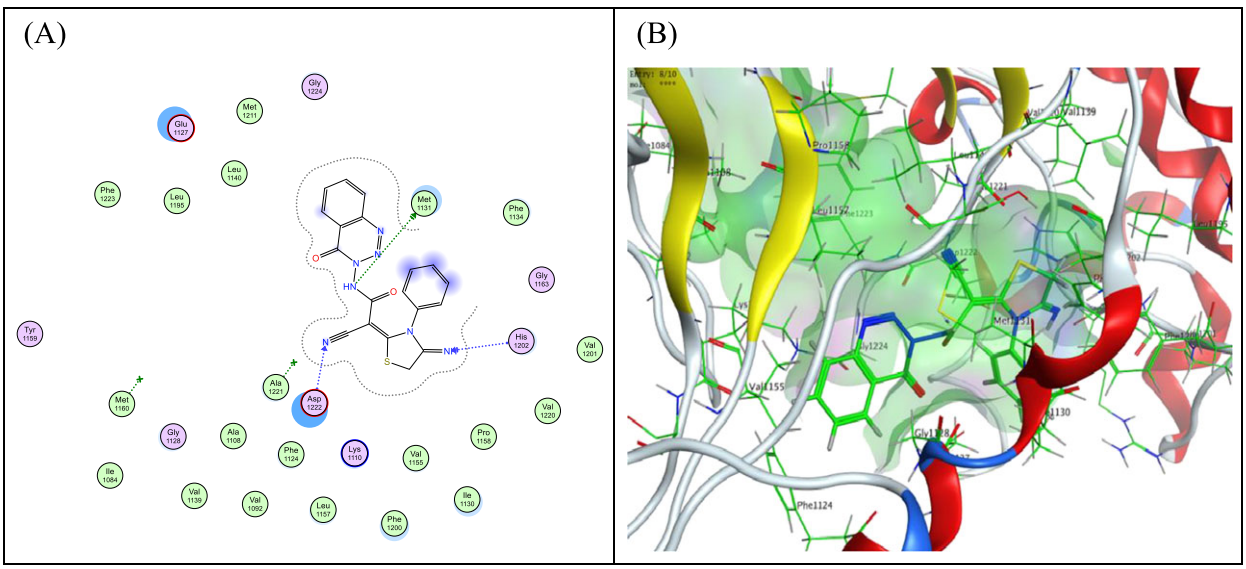


Figure 4. (A) 2D and (B) 3D ligand interaction of **25** with the active sides amino acid of C-Met kinase (S = −1.9895) in comparison with native ligands. [Color figure can be viewed at wileyonlinelibrary.com]

MET1131 (3.77 A˚). It is noteworthy that **7b** revealed a good binding energy score better than the native ligand (S = −22.1657 kcal/mol) (Fig. 2).

Compound **15** formed two hydrogen bonds, one between the N=N group on the triazine ring and GLY1224 (2.98A˚) and the other between the C=O of the amidic group and ASP1222 (1.7A˚). It is noteworthy that **15** revealed a good binding energy score compared with the native ligand (S = −22.1657 kcal/mol) (Fig. 3). Furthermore, compound **25** showed good interactions with the active site amino acids of C-Met kinase (S = −1.9895 kcal/mol) (Fig. 4). The observed binding was mediated by a network of interactions between **25** and C-Met kinase where the NH of the amidic group formed hydrogen bond with MET1131 (3.37 A ˚). Besides, the imine group (C=N) of the thiazole ring displayed hydrogen bond with HIS1202 (3.40 A ˚). Finally, a hydrogen bond was observed between the CN group and ASP1222 (3.31 A ˚). Comparing **15** with **25** could lead to a conclusion that the introduction of the thiazole ring to the backbone of the benzotriazine ring system in case of **25** demonstrated additional advantages by acting as a platform for hydrogen bond. In conclusion, the molecular docking was used to give a preliminary impression of the possible underlying mechanism of action of some promising newly synthesized compounds.

CONCLUSION

This paper reports on the synthesis of various heterocycles incorporating 1,2,3-benzotriazine-4-one moiety in order to evaluate their anticancer activity. The key 2-cyano-*N*-(4-oxobenzo [*d*][1,2,3]triazin-3(4*H*)-yl) acetamide (**5**) (which prepared in 77% yield *via* intramolecular diazo-coupling of 2-amino-*N*′-(2-cyanoacetyl)benzohydrazide) was utilized as a precursor for the synthesis of *N*-benzotriazinyl-pyridones **12a**–**d**, 2-imino-*N*-(benzotriazin-3-yl)-2*H*-chromene derivative **15**, 2-cyano-2-(thiazol-2-ylidene)-*N*-(benzotriazinyl) acetamides **21**, **23**, and **25**, and 5-amino-*N*-(benzotriazinyl)-pyrazoles **29** and **33**. The *in vitro* evaluation of their antitumor activity against liver cells cancer, colorectal cancer, and breast cancer revealed that the benzotriazine-4-one scaffolds **7b**, **15**, and **25** showed the highest antitumor activity with IC_{50} ranging from 5.54 to 16.26 μM.

EXPERIMENTAL

All melting points (uncorrected) were determined on an electrothermal Gallenkamp apparatus (Weiss-Gallenkamp, Loughborough, UK). The IR spectra were measured on a Thermo Scientific Nicolet iS10 FTIR spectrometer (Waltham, MA). ^{1}H NMR spectra were recorded in DMSO-d_6 on Bruker WP spectrometer (Rheinstetten, Germany) at 400 MHz using *TMS* as an internal standard. The mass spectra were recorded on a Quadrupole GC/MS Thermo Scientific Focus/DSQII (Waltham, MA) at 70 eV. Elemental analyses (C, H, and N) were determined on Perkin-Elmer 2400 analyzer (PerkinElmer Instruments, Shelton, CT).

Synthesis of 2-amino-*N*′-(2-cyanoacetyl) benzohydrazide (3). To a suspension of isatoic anhydride (**2**) (0.05 mol, 8.15 g) in absolute ethanol (50 mL), cyanoacetic hydrazide (**1**) (0.05 mol, 5.0 g) was added and then refluxed for 4 h. The precipitate that formed on cooling was filtered to separate the desired product **3**.

White crystals, yield = 67%, mp = 173–175°C. IR ($\bar{\nu}$/cm^{-1}): 3459, 3363, 3250 (NH_2 and NH), 2261 (C ≡ N), 1687, 1654 (C=O). ^{1}H NMR (δ/ppm): 3.78 (s, 2H, CH_2), 6.40 (s, 2H, NH_2), 6.50 (t, 1H, Ar-H), 6.74 (d, 1H, Ar-H), 7.21 (t, 1H, Ar-H), 7.53 (d, 1H, Ar-H), 10.16 (s, 2H, 2NH). Analysis for $C_{10}H_{10}N_4O_2$ (218.08): Calcd: C, 55.04; H, 4.62; N, 25.68%. Found: C, 55.22; H, 4.70; N, 25.77%.

Synthesis of 3-(2-cyanoacetamido)-4-oxo-3*H*-benzo [*d*] [1,2,3]-triazine (5). Sodium nitrite solution (0.7 g in 15 mL H_2O) was added drop by drop to a stirred suspension of benzohydrazide **3** (0.01 mol, 2.18 g) in 5 mL conc. HCl at 0–5°C. The reaction mixture was stirred for an hour at 0–5°C, and the solid product that was obtained by filtration was recrystallized by heating in ethyl alcohol.

Buff crystals, yield = 77%, mp = 195–197°C. IR ($\bar{\nu}$/cm^{-1}): 3174 (NH), 2260 (C ≡ N), 1723, 1693 (C=O). ^{1}H NMR (δ/ppm): 4.17 (s, 2H, CH_2), 8.02–8.33 (m, 4H, Ar-H), 12.32 (s, 1H, NH). MS (EI): *m*/*z* (%) = 229 (molecular ion, 12), 201 (14), 185 (32), 147 (27), 134 (base peak, 100.0), 105 (26), 76 (47), 51 (12). Analysis for $C_{10}H_7N_5O_2$ (229.06): Calcd: C, 52.40; H, 3.08; N, 30.56%. Found: C, 52.52; H, 3.03; N, 30.64%.

Synthesis of 3-(2-cyano-2-arylhydrazono-acetamido)-4-oxo-4*H*-benzo [*d*][1–3] triazines 7a–c. To a cold suspension (0–5°C) of *p*-substituted aniline (0.005 mol) in 1.5 mL conc. HCl $NaNO_2$ solution (0.35 g in 5 mL H_2O) was added dropwise with stirring. This freshly obtained diazonium chloride solution was added dropwise to a cold suspension of the 3-cyanoacetamido-benzotriazine derivative **3** (0.005 mol, 1.14 g) in 25 mL ethanol and 1.5 g of sodium acetate. The stirring time was continued at 0–5°C for additional an hour, and the solid product that collected by filtration was recrystallized by heating in ethyl alcohol.

3-(2-Cyano-2-(phenylhydrazono)-acetamido)-4-oxo-4H-benzo [d][1,2,3] triazine (7a). Yellow crystals, yield = 56%, mp = 238–240°C. IR ($\bar{\nu}$/cm^{-1}): 3339, 3231

(NH), 2216 (C ≡ N), 1692 (broad, C=O). ^{1}H NMR (δ/ppm): 7.18–7.62 (m, 5H, Ar-H), 8.05–8.32 (m, 4H, Ar-H), 11.48 (s, 1H, NH), 12.26 (s, 1H, NH). MS (EI): *m*/*z* (%) = 333 (molecular ion, 31), 208 (base peak, 100.0), 180 (44), 152 (74), 119 (21), 91 (17), 76 (33), 51 (16). Analysis for $C_{16}H_{11}N_7O_2$ (333.10): Calcd: C, 57.66; H, 3.33; N, 29.42%. Found: C, 57.78; H, 3.41; N, 29.55%.

3-(2-Cyano-2-(4-tolylhydrazono)-acetamido)-4-oxo-4H-benzo [d][1,2,3] triazine (7b). Yellow crystals, yield = 77%, mp = 258–260°C. IR ($\bar{\nu}$/cm^{-1}): 3327, 3230 (NH), 2214 (C ≡ N), 1704, 1682 (C=O). ^{1}H NMR (δ/ppm): 2.31 (s, 3H, CH_3), 7.24 (d, 2H, Ar-H), 7.69 (d, 2H, Ar-H), 8.02–8.37 (m, 4H, Ar-H), 12.04 (s, 1H, NH), 12.31 (s, 1H, NH). Analysis for $C_{17}H_{13}N_7O_2$ (347.11): Calcd: C, 58.79; H, 3.77; N, 28.23%. Found: C, 58.63; H, 3.69; N, 28.11%.

3-(2-Cyano-2-(4-anisylhydrazono)-acetamido)-4-oxo-4H-benzo [d][1,2,3] triazine (7c). Brown crystals, yield = 65%, mp = 230–232°C. IR ($\bar{\nu}$/cm^{-1}): 3328, 3230 (NH), 2210 (C ≡ N), 1683 (broad, C=O). ^{1}H NMR (δ/ppm): 3.77 (s, 3H, OCH_3), 6.99 (d, 2H, Ar-H), 7.72 (d, 2H, Ar-H), 8.03 (t, 1H, Ar-H), 8.20 (t, 1H, Ar-H) 8.28–8.36 (m, 2H, Ar-H), 12.06 (s, 1H, NH), 12.33 (s, 1H, NH). Analysis for $C_{17}H_{13}N_7O_3$ (363.11): Calcd: C, 56.20; H, 3.61; N, 26.99%. Found: C, 56.35; H, 3.69; N, 26.93%.

Synthesis of 2-cyano-*N*-(4-oxobenzo [*d*][1,2,3]triazin-3(4*H*)-yl)-3-phenylacrylamide derivatives 9a–d. To a solution of 3-cyanoacetamido-benzotriazine derivative **5** (0.005 mol, 1.14 g) in 15 mL ethanol, 0.005 mol of different substituted benzaldehyde **8** (namely, *p*-tolualdehyde, *p*-anisaldehyde, *p*-chlorobenzaldehyde, and *p*-hydroxybenzaldehyde), and five drops of piperidine were added. The reaction mixture was refluxed for 2 h, and the solid that formed on cooling was filtered and dried.

2-Cyano-N-(4-oxobenzo [d][1,2,3]triazin-3(4H)-yl)-3-(4-tolyl)-acrylamide (9a). Beige crystals, yield = 62%, mp = 290–292°C. IR ($\bar{\nu}$/cm^{-1}): 3287 (NH), 2123 (C ≡ N), 1682 (C=O). ^{1}H NMR (δ/ppm): 2.35 (s, 3H, CH_3), 7.31–8.26 (m, 8H, Ar-H), 9.04 (s, 1H, olefinic CH=C), 11.12 (s, 1H, NH). MS (EI): *m*/*z* (%) = 331 (molecular ion, 18), 208 (base peak, 100), 180 (88), 152 (72), 150 (17), 121 (45), 76 (31), 51 (6). Analysis for $C_{18}H_{13}N_5O_2$ (331.11): Calcd: C, 65.25; H, 3.95; N, 21.14%. Found: C, 65.42; H, 3.99; N, 21.23%.

3-(4-Anisyl)-2-cyano-N-(4-oxobenzo [d][1,2,3]triazin-3(4H)-yl)-acrylamide (9b). Yellow crystals, yield = 55%, mp = 290–292°C. IR ($\bar{\nu}$/cm^{-1}): 3274 (NH), 2205 (C ≡ N), 1661 (C=O). ^{1}H NMR (δ/ppm): 3.87 (s, 3H, OCH_3), 7.14 (d, 2H, Ar-H), 7.95–8.35 (m, 6H, Ar-H), 9.16 (s, 1H, olefinic CH=C), 11.68 (s, 1H, NH). Analysis for $C_{18}H_{13}N_5O_3$ (347.10): Calcd: C, 62.24; H, 3.77; N, 20.16%. Found: C, 62.35; H, 3.79; N, 20.22%.

3-(4-Chlorophenyl)-2-cyano-N-(4-oxobenzo [d][1,2,3]triazin-3(4H)-yl) acrylamide (9c). Pale brown crystals, yield = 65%, mp = 283–285°C. IR ($\bar{\nu}$/cm^{-1}): 3265 (NH), 2123 (C ≡ N), 1692 (C=O). ^{1}H NMR (δ/ppm): 7.35–8.18 (m, 8H, Ar-H), 8.86 (s, 1H, olefinic CH=C), 11.46 (s, 1H, NH). Analysis for $C_{17}H_{10}N_5O_2Cl$ (351.05): Calcd: C, 58.05; H, 2.87; N, 19.91%. Found: C, 58.17; H, 2.95; N, 19.98%.

2-Cyano-3-(4-hydroxyphenyl)-N-(4-oxobenzo [d][1,2,3] triazin-3(4H)-yl) acrylamide (9d). Yellow crystals, yield = 57%, mp = 293–295°C. IR ($\bar{\nu}$/cm^{-1}): 3221 (NH), 2208 (C ≡ N), 1690 (C=O). ^{1}H NMR (δ/ppm): 6.88 (d, 2H, Ar-H), 7.55–8.25 (m, 6H, Ar-H), 8.71 (s, 1H, olefinic CH=C), 9.26 (s, 1H, OH), 11.78 (s, 1H, NH). Analysis for $C_{17}H_{11}N_5O_3$ (333.09): Calcd: C, 61.26; H, 3.33; N, 21.01%. Found: C, 61.17; H, 3.24; N, 20.91%.

Synthesis of 6-amino-4-aryl-2-oxo-1-(4-oxobenzo [*d*][1,2,3] triazin-3(4*H*)-yl)-1,2-dihydropyridine-3,5-dicarbonitrile derivatives 12a–d. **Method A**. A mixture of **9** (0.002 mol) and malononitrile (**10**) (0.002 mol, 0.13 g) has been refluxed for 2 h in 20 mL ethanol containing five drops of piperidine. The precipitate that separated on cooling to room temperature was filtered and dried.

Method B. To a solution of 3-cyanoacetamido-benzotriazine derivative **5** (0.002 mol, 0.46 g) in 15 mL ethanol, different 2-arylidene-malononitrile derivatives **13** (0.002 mol) and five drops piperidine were added. The reaction mixture was boiled under reflux for 2 h, and the precipitate that formed was filtered to give the desired pyridine derivatives **12**.

6-Amino-2-oxo-1-(4-oxobenzo [d][1,2,3]triazin-3(4H)-yl)-4-(4-tolyl)-1,2-dihydropyridine-3,5-dicarbonitrile (12a). Pale yellow crystals, yield = 48%, mp = 278–280°C. IR ($\bar{\nu}$/cm^{-1}): 3424, 3365 (NH_2), 2205 (C ≡ N), 1676, 1625 (C=O). ^{1}H NMR (δ/ppm): 2.40 (s, 3H, CH_3), 7.23–7.38 (m, 6H, Ar-H, and NH_2), 7.89 (t, 1H, Ar-H), 8.05 (t, 1H, Ar-H), 8.19–8.26 (m, 2H, Ar-H). Analysis for $C_{21}H_{13}N_7O_2$ (395.11): Calcd: C, 63.79; H, 3.31; N, 24.80%. Found: C, 63.66; H, 3.25; N, 24.71%.

6-Amino-4-(4-anisyl)-2-oxo-1-(4-oxobenzo [d][1,2,3]triazin-3(4H)-yl)-1,2-dihydropyridine-3,5-dicarbonitrile (12b). Greenish yellow crystals, yield = 50%, mp = 283–285°C. IR ($\bar{\nu}$/cm^{-1}): 3431, 3366 (NH_2), 2204 (C ≡ N), 1676, 1627 (C=O). ^{1}H NMR (δ/ppm): 3.82 (s, 3H, OCH_3), 6.94 (d, 2H, Ar-H), 7.22 (s, 2H, NH_2), 7.54 (d, 2H, Ar-H), 7.91 (t, 1H, Ar-H), 8.04 (t, 1H, Ar-H), 8.21–8.28 (m, 2H, Ar-H). Analysis for $C_{21}H_{13}N_7O_3$ (411.11): Calcd: C, 61.31; H, 3.19; N, 23.83%. Found: C, 61.44; H, 3.30; N, 23.97%.

6-Amino-4-(4-chlorophenyl)-2-oxo-1-(4-oxobenzo [d][1,2,3] triazin-3(4H)-yl)-1,2-dihydropyridine-3,5-dicarbonitrile (12c). Brown powder, yield = 45%, mp = 288–290°C. IR ($\bar{\nu}$/cm^{-1}): 3335, 3196 (NH_2), 2206 (C ≡ N), 1674 (C=O). ^{1}H NMR (δ/ppm): 7.22–7.42 (m, 6H, Ar-H and NH_2), 7.87 (t, 1H, Ar-H), 8.06 (t, 1H, Ar-H), 8.18–8.30 (m, 2H, Ar-H). Analysis for $C_{20}H_{10}N_7O_2Cl$ (415.06): Calcd: C, 57.77; H, 2.42; N, 23.58%. Found: C, 57.89; H, 2.51; N, 23.67%.

6-Amino-4-(4-hydroxyphenyl)-2-oxo-1-(4-oxobenzo [d][1,2,3]triazin-3(4H)-yl)-1,2-dihydropyridine-3,5-dicarbonitrile (12d). Beige crystals, yield = 43%, mp > 300°C. IR ($\bar{\nu}$/cm^{-1}): 3424, 3365, 3152 (NH_2 and OH), 2205, 2182 (C ≡ N), 1677, 1625 (C=O). ^{1}H NMR (δ/ppm): 6.86 (d, 2H, Ar-H), 7.16 (s, 2H, NH_2), 7.44 (d, 2H, Ar-H), 7.86 (t, 1H, Ar-H), 8.02 (t, 1H, Ar-H), 8.18–8.26 (m, 2H, Ar-H), 9.32 (s, 1H, OH). Analysis for $C_{20}H_{11}N_7O_2$ (397.09): Calcd: C, 60.45; H, 2.79; N, 24.68%. Found: C, 60.57; H, 2.91; N, 24.75%.

Synthesis of 2-imino-*N*-(4-oxobenzo [*d*][1,2,3]triazin-3(4*H*)-yl)-2*H*-chromene-3-carboxamide (15). To a suspension of 3-cyanoacetamido-benzotriazine derivative **5** (0.002 mol, 0.45 g) in 15 mL ethyl alcohol, salicylaldehyde (**14**) (0.002 mol, 0.24 mL) and five drops of piperidine were added and refluxed for 2 h. The solid that was obtained on cooling was picked up by filtration.

Beige crystals, yield = 53%, mp = 210–212°C. IR ($\bar{\nu}$/cm^{-1}): 3442 (NH), 1709, 1650 (C=O). ^{1}H NMR (δ/ppm): 6.99–8.59 (m, 8H, Ar-H), 9.48 (s, 1H, chromene H4). Analysis for $C_{17}H_{11}N_5O_3$ (333.09): Calcd: C, 61.26; H, 3.33; N, 21.01%. Found: C, 61.37; H, 3.42; N, 21.12%.

Synthesis of 4,6-dimethyl-2-oxo-1-(4-oxobenzo [*d*][1,2,3]triazin-3(4*H*)-yl)-1,2-dihydropyridine-3-carbonitrile (17). A mixture of 3-cyanoacetamido-benzotriazine derivative **5** (0.002 mol, 0.46 g) and acetyl acetone (**16**) (0.002 mol, 0.2 mL) was refluxed in 15 mL ethyl alcohol and drops of triethylamine for 4 h. The mixture was cooled to 25°C and the precipitate that formed was filtered and dried.

Orange crystals, yield = 52%, mp = 250–252°C. IR ($\bar{\nu}$/cm^{-1}): 2223 (C ≡ N), 1719, 1681 (C=O). ^{1}H NMR (δ/ppm): 2.26 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 6.25 (s, 1H, pyridine H5), 7.94 (t, 1H, Ar-H), 8.11 (t, 1H, Ar-H), 8.29 (d, 1H, Ar-H), 8.43 (d, 1H, Ar-H). Analysis for $C_{15}H_{11}N_5O_2$ (293.09): Calcd: C, 61.43; H, 3.78; N, 23.88%. Found: C, 61.30; H, 3.70; N, 23.75%.

Synthesis of 2-cyano-*N*-(4-oxobenzo [*d*][1,2,3]triazin-3(4*H*)-yl)-2-(3-phenylthiazolidin-2-ylidene) acetamide derivatives 21, 23, and 25. To a stirred suspension of KOH (0.01 mol, 0.56 g) in 20 mL DMF, the 3-cyanoacetamido-benzotriazine derivative **5** (0.01 mol, 2.30 g) and phenyl isothiocyanate (**18**) (0.01 mol, 1.2 mL) were added. The mixture was allowed to stir for 6 h. Chloroacetone, phenacyl chloride, ethyl bromoacetate, and/or chloroacetonitrile (0.01 mol) were added, and the stirring time was continued for further 4 h. The precipitate that formed on dilution with ice-cold water was collected by filtration and recrystallized by heating in ethyl alcohol.

2-Cyano-2-(4-methyl-3-phenylthiazol-2(3H)-ylidene)-N-(4-oxo-benzo [d][1,2,3]triazin-3(4H)-yl) acetamide (21a). Pale yellow crystals, yield = 45%, mp = 260–262°C. IR ($\bar{\nu}$/cm^{-1}): 3211 (NH), 2194 (C ≡ N), 1712, 1693 (C=O). ^{1}H NMR (δ/ppm): 2.50 (s, 3H, CH_3), 5.20 (s, 1H, thiazole H5), 7.04–8.38 (m, 9H, Ar-H), 10.87 (s, 1H, NH). Analysis for $C_{20}H_{14}N_6O_2S$ (402.09): Calcd: C, 59.69; H, 3.51; N, 20.88%. Found: C, 59.77; H, 3.54; N, 20.95%.

2-Cyano-2-(3,4-diphenylthiazol-2(3H)-ylidene)-N-(4-oxobenzo [d][1,2,3]triazin-3(4H)-yl) acetamide (21b). Yellow crystals, yield = 62%, mp = 198–200°C. IR ($\bar{\nu}$/cm^{-1}): IR ($\bar{\nu}$/cm^{-1}): 3246 (NH), 2194 (C ≡ N), 1692, 1643 (C=O). ^{1}H NMR (δ/ppm): 6.88 (s, 1H, thiazole H5), 7.16–8.36 (m, 14H, Ar-H), 10.97 (s, 1H, NH). Analysis for $C_{25}H_{16}N_6O_2S$ (464.11): Calcd: C, 64.64; H, 3.47; N, 18.09%. Found: C, 64.51; H, 3.41; N, 17.98%.

2-Cyano-2-(4-oxo-3-phenylthiazolidin-2-ylidene)-N-(4-oxobenzo [d][1,2,3]triazin-3(4H)-yl) acetamide (23). Buff crystals, yield = 66%, mp = 277–280°C. IR ($\bar{\nu}$/cm^{-1}): 3320 (NH), 2195 (C ≡ N), 1736, 1712, 1677 (C=O). ^{1}H NMR (δ/ppm): 4.08 (s, 2H, CH_2), 7.18–8.31 (m, 9H, Ar-H), 11.69 (s, 1H, NH). Analysis for $C_{19}H_{12}N_6O_3S$ (404.07): Calcd: C, 56.43; H, 2.99; N, 20.78%. Found: C, 56.31; H, 2.91; N, 20.69%.

2-Cyano-2-(4-imino-3-phenylthiazolidin-2-ylidene)-N-(4-oxobenzo [d][1,2,3]triazin-3(4H)-yl) acetamide (25). Brown crystals, yield = 46%, mp = 230–231°C. IR ($\bar{\nu}$/cm^{-1}): 3434, 3277 (NH), 2189 (C ≡ N), 1699, 1650 (C=O). ^{1}H NMR (δ/ppm): 3.89 (s, 2H, CH_2), 7.32–8.31 (m, 9H, Ar-H), 10.24 (s, 1H, NH), 11.13 (s, 1H, NH). Analysis for $C_{19}H_{13}N_7O_2S$ (403.09): Calcd: C, 56.57; H, 3.25; N, 24.30%. Found: C, 56.66; H, 3.34; N, 24.39%.

Synthesis of 2-cyano-3-(dimethylamino)-*N*-(4-oxobenzo [*d*][1,2,3]-triazin-3(4*H*)-yl) acrylamide (27). To a solution of 3-cyanoacetamido-benzotriazine derivative **5** (0.005 mol, 1.15 g) in boiling dry xylene (15 mL), dimethylformamide dimethylacetal (DMF-DMA) (0.005 mol, 0.56 mL) was added. The reaction mixture was refluxed for 4 h, cooled and diluted with water, and the solid product was filtered and recrystallized by heating in ethyl alcohol.

Pale yellow crystals, yield = 64%, mp = 198–200°C. IR ($\bar{\nu}$/cm^{-1}): 3342 (NH), 2195 (C ≡ N), 1688, 1668 (C=O). ^{1}H NMR (δ/ppm): 3.25 (s, 3H, CH_3), 3.36 (s, 3H, CH_3), 7.87 (s, 1H, olefinic CH=C), 7.98 (t, 1H, Ar-H), 8.15 (t, 1H, Ar-H), 8.28–8.30 (m, 2H, Ar-H), 11.15 (s, 1H, NH). Analysis for $C_{13}H_{12}N_6O_2$ (284.10): Calcd: C, 54.93; H, 4.25; N, 29.56%. Found: C, 54.80; H, 4.19; N, 29.47%.

Synthesis of 5-amino-*N*-(4-oxobenzo [*d*][1,2,3]triazin-3(4*H*)-yl)-1*H*-pyrazole-4-carboxamide (29). To a suspension of enaminonitrile **27** (0.005 mol, 1.42 g) in 20 mL ethyl alcohol, hydrazine hydrate (0.01 mol, 0.50 mL) was added and then boiled under reflux for 2 h. After cooling, the solid product that formed was filtered and dried.

Pale brown crystals, yield = 52%, mp > 300°C. IR ($\bar{\nu}$/cm^{-1}): 3437, 3362, 3206 (NH_2 and NH), 1648 (broad, C=O). ^{1}H NMR (δ/ppm): 7.05 (s, 2H, NH_2), 7.84 (t, 1H, Ar-H), 8.06 (t, 1H, Ar-H), 8.17–8.26 (m, 2H, Ar-H), 8.62 (s, 1H, pyrazole H3), 11.24 (s, 1H, NH), 12.92 (s, 1H, NH). Analysis for $C_{11}H_9N_7O_2$ (271.08): Calcd: C, 48.71; H, 3.34; N, 36.15%. Found: C, 48.88; H, 3.41; N, 36.23%.

Synthesis of 2-cyano-3,3-bis (methylthio)-*N*-(4 oxobenzo [*d*] [1,2,3]-triazin-3(4*H*)-yl) acrylamide (32). To a suspension of solid KOH (0.02 mol, 1.12 g) in DMSO (20 mL), the 3-cyanoacetamido-benzotriazine derivative 5 (0.01 mol, 2.29 g) was added followed by the addition of carbon disulfide (0.02 mol, 1.5 mL). The mixture was stirred overnight, then methyl iodide (0.02 mol, 1.30 mL) was added, and stirring time was continued for additional 4 h. The precipitate that formed, on dilution with ice-cold water, was filtered and recrystallized by heating in ethyl alcohol.

Yellow crystals, yield = 55%, mp = 185–187°C. IR ($\bar{\nu}$/cm^{-1}): 3245 (NH), 2201 (C ≡ N), 1708, 1683 (C=O). ^{1}H NMR (δ/ppm): 2.66 (s, 3H, CH_3), 2.73 (s, 3H, CH_3), 8.01 (t, 1H, Ar-H), 8.18 (t, 1H, Ar-H), 8.30–8.34 (m, 2H, Ar-H), 12.33 (s, 1H, NH). Analysis for $C_{13}H_{11}N_5O_2S_2$ (333.04): Calcd: C, 46.84; H, 3.33; N, 21.01%. Found: C, 46.98; H, 3.35; N, 21.13%.

Synthesis of 3-amino-5-(methylthio)-*N*-(4-oxobenzo [*d*] [1,2,3]-triazin-3(4*H*)-yl)-1*H*-pyrazole-4-carboxamide (33). A mixture of ketene dithioacetal 32 (0.002 mol, 0.66 g) and hydrazine hydrate (0.002 mol, 0.1 mL) was boiled under reflux for 4 h in 15 mL ethyl alcohol. After cooling, the solid that formed was filtered and dried.

Off-white needles, yield = 46%, mp = 290–292°C. IR ($\bar{\nu}$/cm^{-1}): 3443, 3324 (NH_2 and NH), 1620 (broad, C=O). ^{1}H NMR (δ/ppm): 2.61 (s, 3H, CH_3), 7.33 (s, 2H, NH_2), 7.52 (t, 1H, Ar-H), 7.70 (t, 1H, Ar-H), 8.15–8.24 (m, 2H, Ar-H), 10.51 (s, 1H, NH), 11.87 (s, 1H, NH). Analysis for $C_{12}H_{11}N_7O_2S$ (317.07): Calcd: C, 45.42; H, 3.49; N, 30.90%. Found: C, 45.30; H, 3.43; N, 30.82%.

Anticancer screening. The evaluation of *in vitro* cytotoxicity effects of the synthesized 1,2,3-benzotriazine-4-one scaffolds was carried out against three human cancer cell lines (namely, hepatocellular carcinoma HepG-2, colorectal carcinoma HCT-116, and mammary gland breast cancer MCF-7). These cell lines were obtained from ATCC *via* Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. Cytotoxicity determinations are based on the transformation of the yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in practical cells. The method of this MTT test was performed as previously described in detail [19–21].

Docking studies. Molecular docking software attempts to simulate the structure (or structures) of the intermolecular complex formed between two or more molecules, usually a large structure of a protein complexing to its favored small ligand. Docking has been widely used to suggest the binding modes of protein inhibitors [22–25] to the protein active site. Most docking algorithms generate a large number of possible structures, so they require a means to score each structure to identify those of most interest. In general, the scope of docking studies is thus concerned with the generation and evaluation of plausible structures of intermolecular complexes.

This part of the study was aiming to perform virtual screening through molecular docking of the selected compounds in the active site of tyrosine kinase receptor domain of the hepatocyte growth factor receptor C-MET (PDB I.D.:3ce3). Data obtained for the most two active compounds in this study, namely, **8** and **11b**, are described herein. During the docking procedure, the default settings were applied with the aid of MOE v10.2009.

The C-Met kinase protein structure was first repaired and then appropriately protonated in the presence of ligand using the protonate 3D process in MOE and then was used directly for docking. Docking validation was first performed to confirm the appropriateness of the chosen docking parameters, which were found to be the default parameters, through determining the ability of the cocrystallized ligand to accurately identify the binding pocket and also to form similar binding interactions like the ones originally attained in its crystal structure. The completeness of the validation procedure was assessed based on the value of the relative mean standard deviation obtained for the redocked native ligand.

Thereafter, the candidate ligands were prepared by performing a systematic conformational search associated with energy minimization. The lowest energy conformer for each candidate was utilized for performing the docking study. Docking of the investigated new compounds was performed using the default parameters with the aid of triangle matcher placement method with London dG scoring, which was used for the docking runs, and the 10 docking poses retained by the software were individually examined for final choice of the best docking pose. The best docking conformer for each candidate ligand was selected based on a set of parameters, namely, the S score reflecting the ligand's binding energy, energy of placement, energy of conformation, and ligand–receptor binding interactions.

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