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Design, synthesis, and molecular modelling of pyridazinone and phthalazinone derivatives as protein kinases inhibitors

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ABSTRACT

The design and synthesis of pyridazinone and phthalazinone derivatives are described. Newly synthesized compounds were tested on a panel of four kinases in order to evaluate their activity and potential selectivity. In addition, the promising compounds were tested on four cancer cell lines to examine cytotoxic effects. The compounds inhibited DYRK1A and GSK3 with different activity. SAR analysis and docking calculations were carried out to aid in the interpretation of the results. Taken together, our findings suggest that pyridazinone and phthalazinone scaffolds are interesting starting points for design of potent GSK3 and DYRK1A inhibitors.

Large numbers of protein kinases are involved in controlling the phosphorylation of protein in cells. This process plays an important role in the regulation of various cellular processes.[1–3] Alterations of this kinase signalling are found in numerous human pathologies.[4] Accordingly, kinase inhibitors continue to be of high interest for therapeutic intervention. Currently, large numbers of small molecule kinase inhibitors are undergoing clinical trials for the treatment of cancer and other diseases, showing that kinases constitute important therapeutic targets.

The role of cyclin-dependent kinases (CDKs) to regulate the cell cycle and apoptosis have been well explored.[5–8] They are involved in several diseases, including cancer, Alzheimer's disease, Parkinson's disease, stroke, diabetes, polycystic kidney disease, glomerulonephritis, inflammation, and AIDS.[5,9] Glycogen synthase kinase-3 (GSK-3) plays an important role in a large number of cellular processes, apoptosis control, neurodegenerative disorders (Alzheimer's disease) and cardiovascular diseases.[9] Furthermore, casein kinase 1 (CK1) plays an important role in controlling cell differentiation, proliferation, apoptosis, circadian rhythms and has been implicated in neurodegenerative diseases.[10,11] Dual specificity, tyrosine phosphorylation regulated kinase 1A (DYRK1A) plays a key role in Alzheimer's disease and Down syndrome.[12]

Several structurally diverse ligands were previously reported to inhibit GSK-3. Examples include thiadiazolidindiones (TDZD), hydantoins, triazoles, thiazoles, maleimides, dithiazolidindiones, and pyrazolepyridines.[13–19] Many current efforts toward the development of novel kinase inhibitors concentrate on structure-based ligand design. A large number of X-ray structures of kinase-inhibitor complexes are deposited in the Protein Data Bank with resolutions in the range of 1.95–2.8 Å that provide a basis for compound design and optimization. We have explored pyridazinone and phthalazinone scaffolds as potentially novel kinase inhibitors. Herein, we report the design, synthesis and evaluation of novel pyridazinone and phthalazinone derivatives.

The synthesized compounds were tested for kinase inhibitory activity against four protein kinases including DYRK1A, CK1, CDK5, and GSK3 types and, in addition, for cytotoxic activity against four different human cancer cell lines. Structure–activity relationships and the potential contribution of kinase inhibition to anti-tumor properties are discussed.

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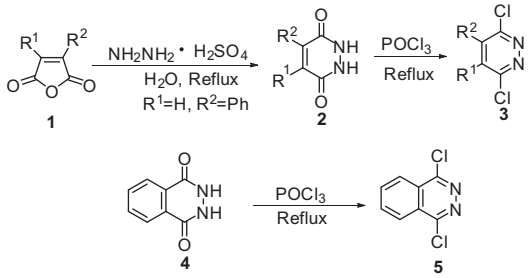
A detailed literature survey revealed that the majority of currently available kinase inhibitor scaffolds consist of planar heterocycles that carry both hydrogen bond donors and acceptor moieties.[20–22] The analysis of cyclin-dependent kinase inhibitors and comparison with pyridazinone and phthalazinone cores suggested that derivatives of these scaffolds should merit consideration as potential kinase inhibitors (Fig. 1). Hence, we synthesized pyridazinone and phthalazinone derivatives and evaluated their kinase inhibitor property.

Heating 3-phenylmaleic anhydride **1** under reflux with hydrazine sulfate in water yielded intermediate **2**.[23,24] Further treatment of **2** with $POCl_3$ gave the dichloro derivatives **3**.[24] Similarly, 1,4-dichlorophthalazine **5**[25] was synthesized from the commercially available 2,3-dihydrophthalazine-1,4-dione **4** by treating with $POCl_3$ (Scheme 1).



Scheme 1. Synthesis of the dichloro derivatives **3** and **5**.

Amination of intermediates **3** and **5** was carried out with various amines (Scheme 2a and b). The use of excess amine with intermediate **3** leads to the formation of two isomers **6a–h**. The two isomers can be separated easily using flash chromatography to give each isomer in pure form except in the cases of **6g** and **6h** where close rf resulted in poor separation and yielded only a small amount of pure **6g**. The use of the microwave was found to accelerate the reaction rate and reduce the reaction time dramatically from 3 h to 30 min.[26] Compounds **7a–g** were obtained by heating **6a–g** with acetic acid in presence of sodium acetate under reflux overnight using conventional heating.[27] The same result was achieved by heating the same reaction mixture in microwave at 120 °C for 5 min.

The use of excess amine in the case of intermediate **5** gives the diamine **8e**. Mono-substitution of intermediate **5** was achieved using an equimolar quantity of the appropriate amine in presence of base to afford **8a–d** as single isomers. Hydrolysis of **8a–d** was achieved using the above mentioned method to afford **9a–d.**

Simple amino substituted compounds **10a**, **10b** and **16** were synthesised by the direct amination of chloro aromatics **3** and **5** with NH_4OH either under reflux overnight or microwave irradiation for 30 min (see Scheme 3a and b).

Hydrolysis of compound **10a** with acetic acid in the presence of sodium acetate for 3 h, gave two products **11a** and **11b**, which

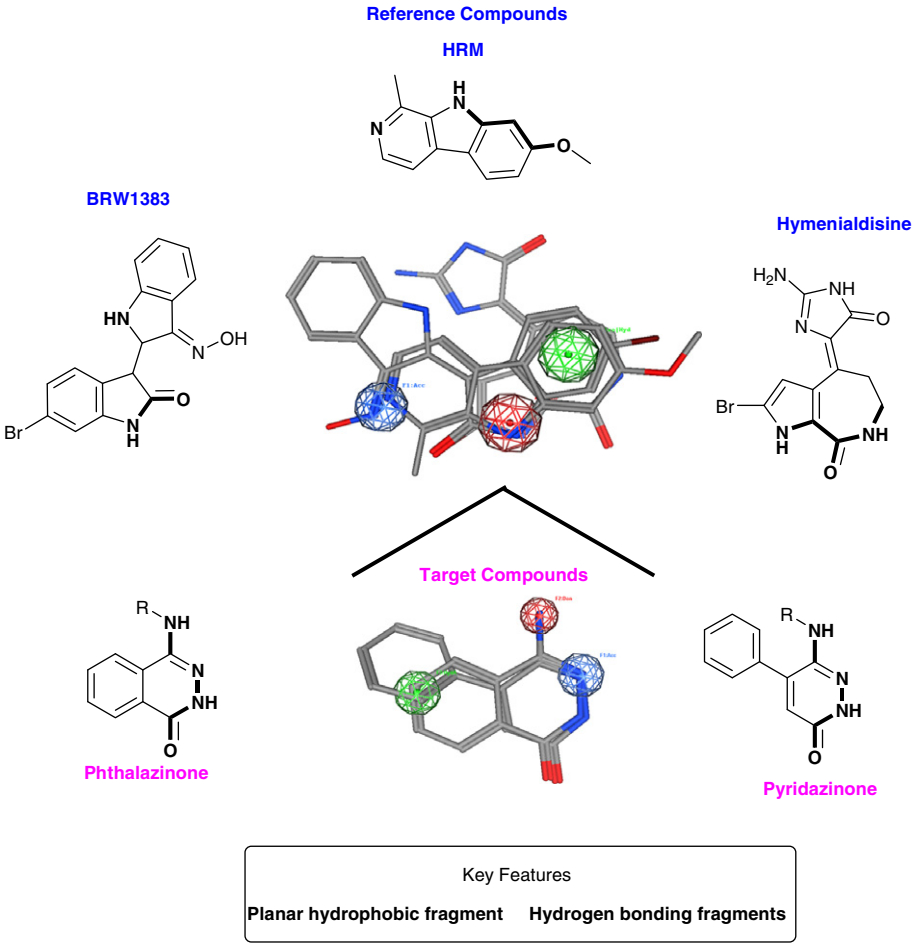
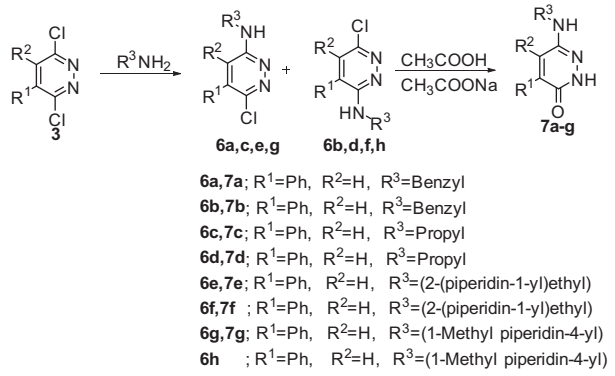


Figure 1. Structural similarity of cyclin-dependent kinase inhibitors and pyridazinone and phthalazinone scaffolds. HRM, a selective DYRK1A inhibitor, hymenialdisine, an inhibitor of different kinases, and BRW1383, a selective GSK3 inhibitor, are compared to pyridazinone and phthalazinone. These compounds display pharmacophoric resemblance. Key structural features are highlighted in red or bold lines.



Scheme 2a. Synthesis of pyridazinone derivatives **7a–g**.

were separated by flash chromatography. By hydrolysis of **10b** with acetic acid under reflux conditions overnight compound **12** was obtained as a single product. Compound **13** was prepared by removal of the acetyl group of **12** using a mixture of $NaHCO_3$ in H_2O-dioxan under reflux for 48 h. Benzoylation of compound **10b** using benzoyl chloride in the presence of DIPEA gave compound **14,** which was hydrolyzed by acetic acid and sodium acetate to produce **15**.

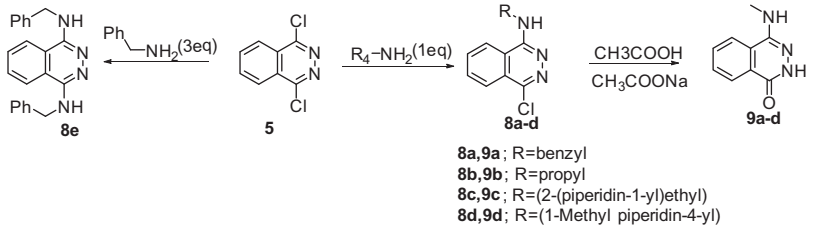
For the phthalazine derivatives, reaction of the NH_2 with acid chlorides or anhydrides using microwave heating gave clean reactions of mixtures of mono, di-substituted amines which can be resolved by hydrolysis to give the desired compounds **20a–d**.

Reaction of compound **16** with acetic acid and sodium acetate produced a mixture of **20c** and **20e**, which was confirmed by HPLC. Heating the resulting reaction mixture with water gave the **20e**. Reaction of compound **16** with benzoyl chloride followed by in situ hydrolysis with acetic acid and sodium acetate produced **20f** in a single step.

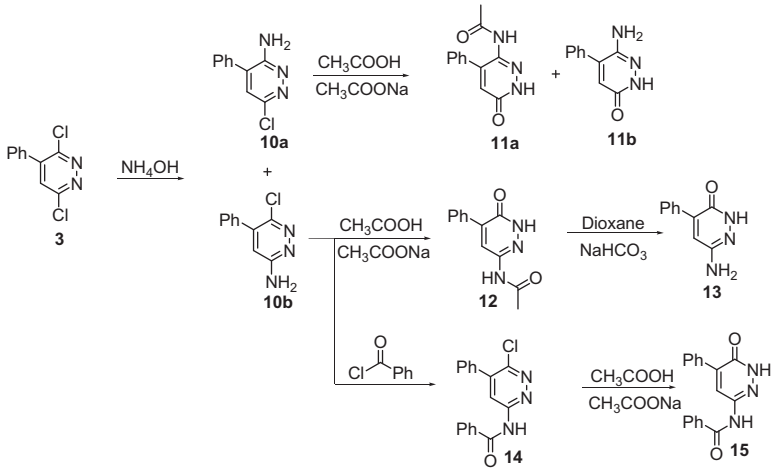
All synthesized compounds were evaluated for their inhibitory activities against different kinases including DYRK1A, CK1, CDK5, and GSK3 (Table 1). Three compounds showed promising initial activity with IC_{50} values ranging from 2.2 to 8.1 μM. Two compounds **12** and **20e** exhibited good differential inhibitory activity against GSK3 and DYRK1A enzymes and displayed a selective tendency. In contrast, compound **13** was active against DYRK1A and GSK. In our study, compounds were considered inactive if IC_{50} values were 10 μM or larger.

The compounds **12**, **13** and **20e** were evaluated against four tumour cell lines, and found to be active (Table 2). All three compounds were active against all four cell lines, with IC_{50} values of less than 15 μM over all the cell lines. The potency of all three compounds against cell line SF-295 was comparable. Furthermore, compound **13** was most potent against all cell lines. Given the activity of **13** for GSK3 and DYRK1A, these kinases might play an important role in these four different types of cancer cells.

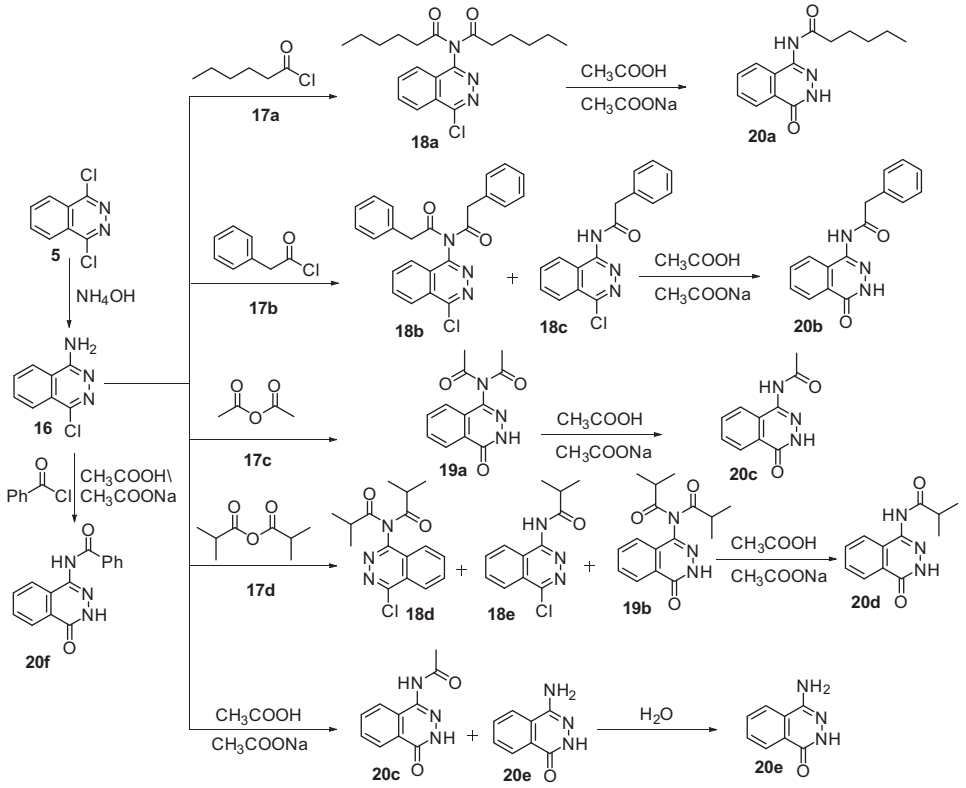
Regarding Kinases activity, only three compounds exhibited promising activity, two phenyl pyridazine and one phthalazine analogs. 5-Substitution of phenyl pyradazine with amino function group results a compound **13** with modest activity (6 and 8.1 μM) towards only DYRKA and GSK-3 kinases respectively. However, acetylation of such compound revealed a potent compound **12** (IC_{50} 2.2 μM) with GSK-3 kinase selectivity and lost of the activity towards the others. This proved that the substitution in the 5-postion of phenyl pyridazine scaffold affected and trapped the activity between only DYRKA and GSK-3 enzymes. The other derivative of phthalazine scaffold **20e** showed a promising degree of activity and hence selectivity (4.1 μM) against DYRKA enzyme through the 4-amino substitution. Taken all together, we can conclude that, (1) all target compounds have only activity against two enzymes from



Scheme 2b. Synthesis of phthalazinone derivatives **9a–d**.



Scheme 3a. Synthesis of pyridazinone substituted amide **11–15**.



Scheme 3b. Synthesis of phthalazinone substituted amide **20a–f**.

Table 1
Kinases activity

Compound	IC_{50} (μM)*			
	DYRK1A	CK1	CDK5	GSK3
7a–g	>10	>10	>10	>10
9a–d	>10	>10	>10	>10
11a–b	>10	>10	>10	>10
12	10	>10	>10	2.2
13	6	>10	>10	8.1
14	>10	>10	>10	>10
15	>10	>10	>10	>10
18a–c	>10	>10	>10	>10
19a–b	>10	>10	>10	>10
20a–d	>10	>10	>10	>10
20e	4.1	>10	>10	>10
20f	>10	>10	>10	>10

* All measurements were determined in triplicate, and mean values are reported. The standard error of determination in all cases does not exceed 10%. IC50 value reported as >10 μM indicates that the compound did not display any inhibitory activity at the highest concentration tested (10 μM).

Table 2
Cytotoxic activity of compounds **12**, **13** and **20e** against four cell lines

Compounds	Cancer cell lines (IC_{50}, μM)*			
	OVCAR-8	SF-295	HCT-116	HL-60
12	14.00	7.66	15.44	11.61
13	4.86	5.71	4.40	3.64
20e	11.78	6.06	12. 71	8.87

* All measurements were determined in triplicate, and mean values are reported. Data are presented as IC_{50} values, with 95% confidence intervals obtained by non-linear regression for all cell lines.

the kinase panel, (2) two factors affect activity/selectivity towards these enzymes, that is, scaffolds and distinct substitution patterns. In addition, molecular docking was carried out for further SAR analysis.

To explore possible SAR characteristics of the newly identified inhibitors, docking analysis was carried out using the Molecular Operating Environment (MOE 2008.10)[28] on the basis of high resolution crystal structures of DYRK1A in complex with HRM (PDB code 3ANR) and GSK3 in complex with BRW1383 (PDB code 1UV5).[29] Target proteins and ligands were energy-minimized using MOE. The following parameters were used for energy minimization; gradient: 0.01, force field: MMFF94X, chiral constraint: current geometry; total runs = 50, cycles/run = 15, iteration limit = 10,000, potential energy grid: on, annealing algorithm: simulated annealing. We compared newly active compounds to crystallographic reference inhibitors to derive hypothetical binding modes and study structural features implicated in activity. Figures 2 and 3 show the crystallographic binding modes of these two inhibitors. First, crystallographic inhibitors were redocked to reproduce their structure and adjust docking parameters accordingly. Only 6-amino phenyl pyradazinone **13** was active against DYRK1A and GSK3. Introduction of an acetyl amino group instead of the amine rendered the compound **12** selective for GSK3.

Hence, modifications at this site modulated the activity of pyradazinone ligands. The new inhibitors were docked into the active sites of DYRK1A and GSK3. Figures 4 and 5 show preferred docking poses of compound **13** in complex with DYRK1A and GSK3.

In addition, Figure 6 shows the hypothetical binding mode of compound **12** in the active site of GSK3 and Figure 7 the hypothetical binding mode of compound **20e** in the active site of DYRK1A. From the docking studies, preliminary SAR trends are beginning to emerge.

The binding mode of the crystallographic inhibitor HRM and the putative binding mode of compound **13** in the active site of DYRK1A are very similar involving interactions with Leu241 and Glu239. As indicated in Figure 8, compound **20e** may form three

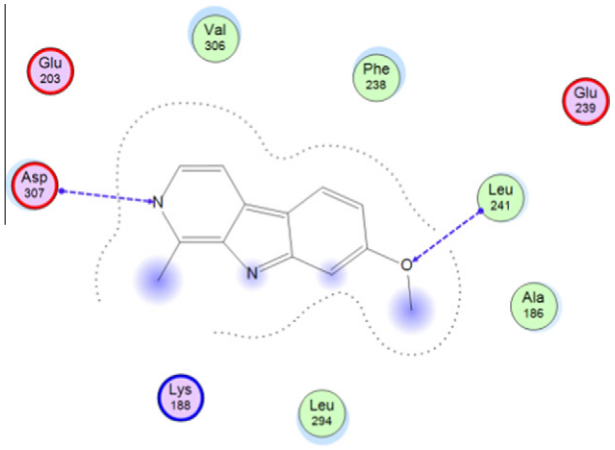


Figure 2. Crystallographic binding mode of HRM in the active site of DYRK1A (PDB code 3ANR).

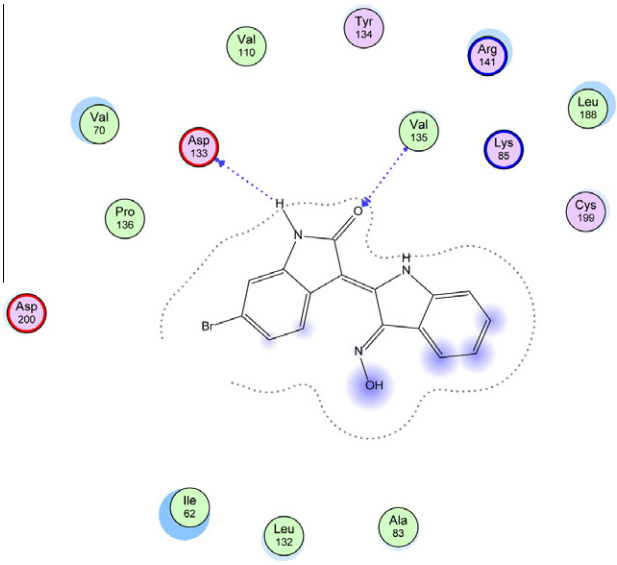


Figure 3. Crystallographic binding mode of BRW1383 in the active site of GSK3 (PDB code 1UV5).

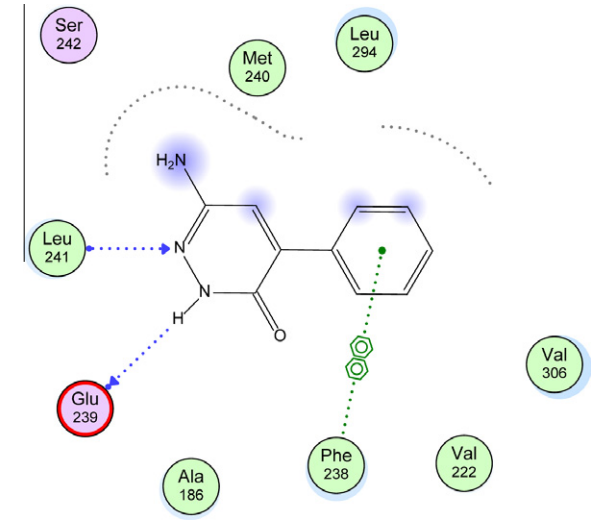


Figure 4. Hypothetical binding mode of compound **13** in the active site of DYRK1A.

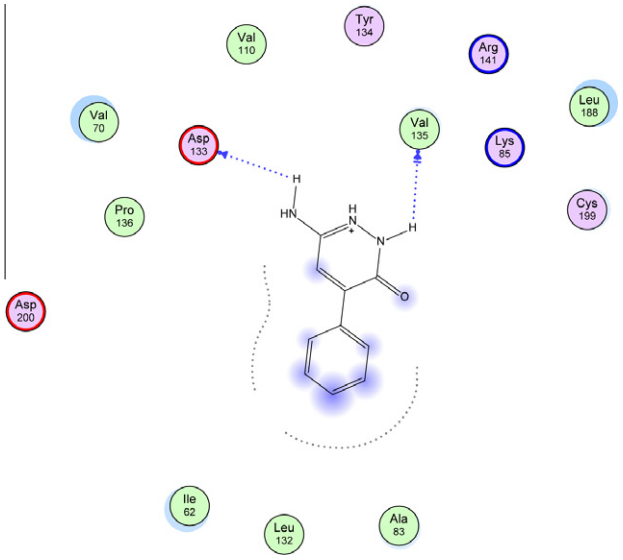


Figure 5. Hypothetical binding mode of compound **13** in the active site of GSK3.

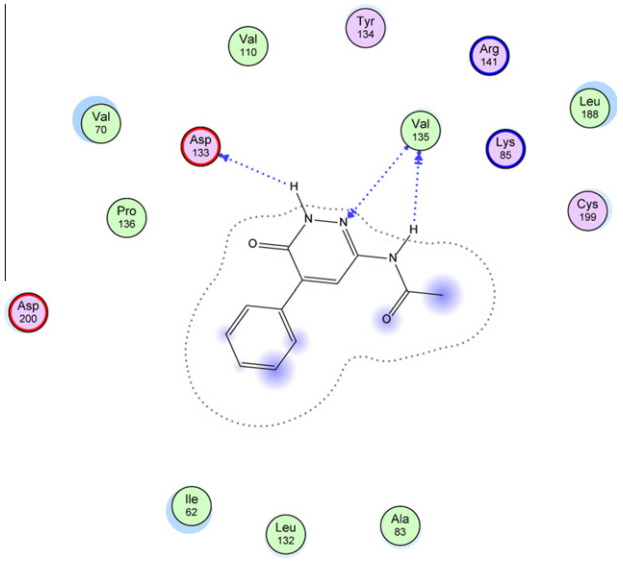


Figure 6. Hypothetical binding mode of compound **12** in the active site of GSK3.

hydrogen bonding interactions via the amino group substituent and, in addition, aromatic/hydrophobic interaction with the Leu241 residue. This binding mode may results in a selective tendency, although these interactions cannot be predicted with certainty. However, these putative interactions are quite different from the reference ligand Figure 2 that may contribute to selectivity towards DYRK1A. Similar observations are made for compound **13** and crystallographic inhibitor BRW1383 in the active site of GSK3, Figure 5. Taken together, these observations might rationalize the observed dual activity of compound **13**. Also, the putative interaction of compound **12** with GSK3 suggests that the pyradazine ring might form two hydrogen bond interactions with the backbone carbonyl group of Asp133 and the backbone amide of Val135 respectively. Moreover, another hydrogen bond might be formed through the acetamido NH with Val135 residue, different from binding mode of compound **13** in Figure 6. In addition, the fit of the aromatic ring into the hydrophobic pocket might support selective binding, in addition to the orientation of the acetamido group relative to the imide N in the inodolinoxime fragment of the BRW reference compound in Figure 9.

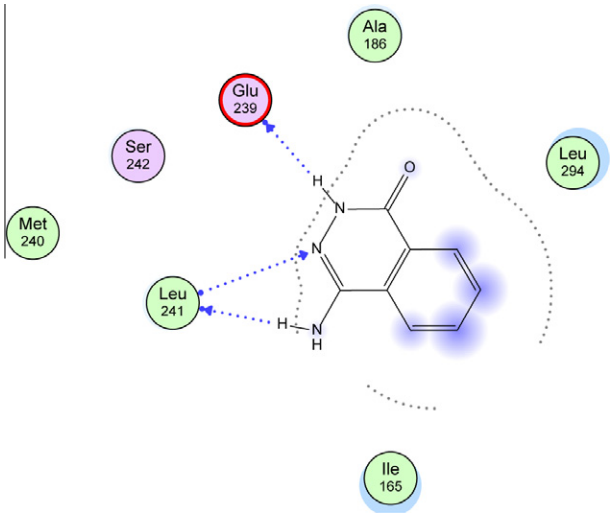


Figure 7. Hypothetical binding mode of compound **20e** in the active site of DYRK1A.

Figure 8. 2D Interaction model of complex HRM-DYRK1A. Ligands HRM (pink), **20e** (green), and **13** (blue) are docked and shown with the residues of active site. Hydrogen bonds are shown as dotted lines consistent with ligand colors.

In conclusion, we have designed and synthesized a new series of bicyclic heteroaromatic systems of fused or linked types containing pyridazinone fragment. Structure–activity relationships of these compounds and cellular activities have been explored. Different activity patterns were identified and investigated for three of these inhibitors and kinases DYRK1A and GSK3. Dual and single kinases inhibitors **13**, **12**, and **20e** were identified and will be used as starting potential hits for further optimization.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.02.027.

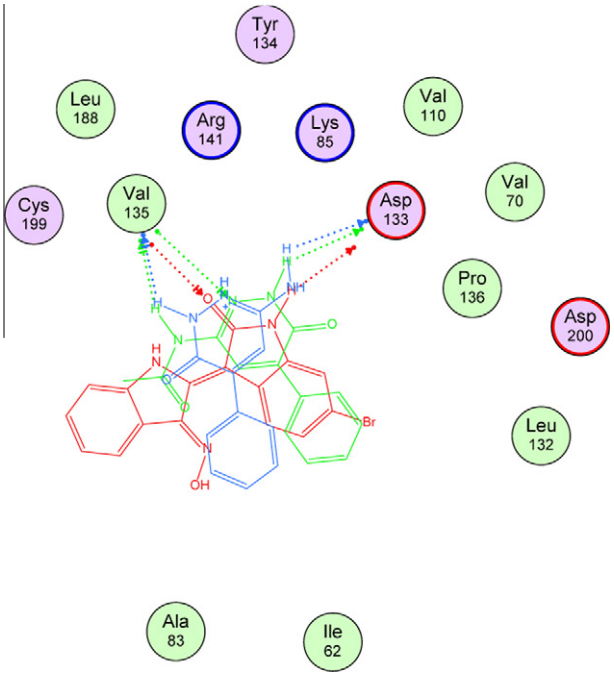

Figure 9. 2D Interaction model of complex BRW-GSK3. Ligands BRW (red), **12** (green), and **13** (blue) are docked and shown with the residues of active site. Hydrogen bonds are shown as dotted lines consistent with ligand colors.

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