Bioorganic & Medicinal Chemistry Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of phenylpiperazine derivatives as IGF-1R inhibitor with potent antiproliferative properties in vitro

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ARTICLE INFO

Article history:
Received 4 November 2014
Revised 18 December 2014
Accepted 6 January 2015
Available online xxxx

Keywords:
Phenylpiperazine
IGF-1R inhibitors
Apoptosis
Computer simulation

ABSTRACT

A series of phenylpiperazine derivatives (**3a**–**3q**) were designed and synthesized. In vitro assays indicated that several phenylpiperazine derivatives had excellent antiproliferative properties against four cancer cell lines including multidrug-resistant cancer cell lines, with IC_{50} values in the low micromolar range. The average IC_{50} of the most active compound **3b** is 0.024 μM to the MCF-7 cell line. In addition, the mechanism of action of these new analogues was investigated by molecular docking studies, insulin-like growth factor 1-receptor (IGF-1R) kinase assay and apoptosis induced assay. These studies confirmed that these new phenylpiperazine derivatives maintain their mechanisms of action by disrupting IGF-1R kinase.

The insulin-like growth factor 1 receptor (IGF-1R), as part of the large class of receptor tyrosine kinases (RTKs), is now considered a potential cellular oncogene that plays a key role in various cellular processes, such as proliferation, survival, transformation, differentiation as well as cell-cell and cell-substrate interactions.[1,2] Signaling through IGF-1R is initiated upon the binding of the IGF-I ligand to the receptor leading to receptor dimerization, autophosphorylation, and subsequent activation of the downstream substrates Shc, IRS-1, and IRS-2.[3]

It has been demonstrated that IGF-1R expression is a fundamental prerequisite for cellular transformation because enhanced IGF-1R levels and IGF-I signaling are considered key factors for the cell to adopt the proliferative and oncogenic pathways.[4] From a clinical perspective, epidemiological studies have correlated elevated IGF-I levels with increased risk of developing colon, breast, prostate, and lung tumors, highlighting the importance of IGF-1R signaling.[5–10] In breast neoplastic cell lines, expression of IGF-1R is a fundamental prerequisite for a malignant phenotype, potentially facilitating cell survival and metastasis.[11–14] For these reasons, the IGF-1R has emerged as a therapeutic target for the treatment of human cancer.

Bristol–Myers Squibb (BMS) company discovered 1*H*-benzoimidazol-2-yl)-1*H*-pyridin-2-one (BMS-536924) (Fig. 1A) as a novel ATP-competitive inhibitor of IGF-1R.[3] We can clearly know that the compound contains the structure of phenyl ethanol. Velaparthi et al.[15] designed and synthesized a series of new compounds that piperazine replacements of morpholine in BMS-536924 (Fig. 1B). With the participated of the piperazine ring, some of the IGF-1R inhibitory activity were much better than BMS-536924. Girnita et al.[16] used molecular modeling showed that a molecule consisting of two benzene rings separated by only one carbon atom could mimic the suggested three-dimensional structure of the two tyrosines of IGF-1R, and thereby possibly inhibits their phosphorylation. We therefore attempted to synthesis a series of phenylpiperazine derivatives as novel IGF-1R inhibitors with 1-bromophenyl ethanol as the skeleton which could be beneficial to patients suffering from various cancers.

Subsequently, in order to validate whether these designed compounds can work on target protein IGF-1R, the molecular docking was performed by fitting these designed compounds and reference compound (BMS-536924) into the ATP binding site of IGF-1R. (PDB code: 2OJ9).[17] Then, the obtained results have been plotted as a line-scatter graph and presented in Figure 2, which mainly displays the corresponding CDOCKER_INTERACTION_ENERGY of the molecular docking studies.[18] Compared with the positive drug BMS-536924, it was clearly seen that compounds **3b** and **3a** showed lower interaction energy than positive that reached up to −53.41 kcal/mol and −52.17 kcal/mol, respectively. Besides, almost all the designed molecules possessed low interaction energy, demonstrating that they are likely to exhibit potent inhibitory activity against IGF-1R tyrosine kinase. Therefore, this preliminary analysis served as a modest stimulant to induce us to synthesize these 1-(4-bromophenyl)-2-(4-phenylpiperazin-1-yl)ethanol compounds.

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http://dx.doi.org/10.1016/j.bmcl.2015.01.011
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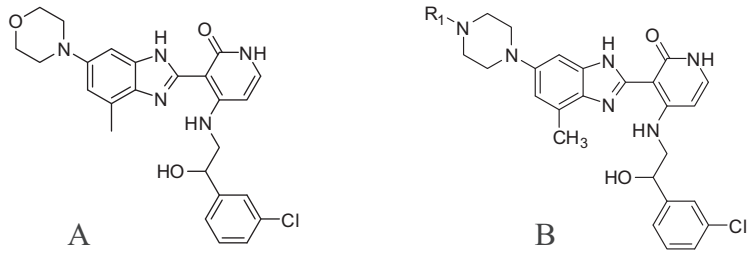


Figure 1. The structure of compounds A and B.

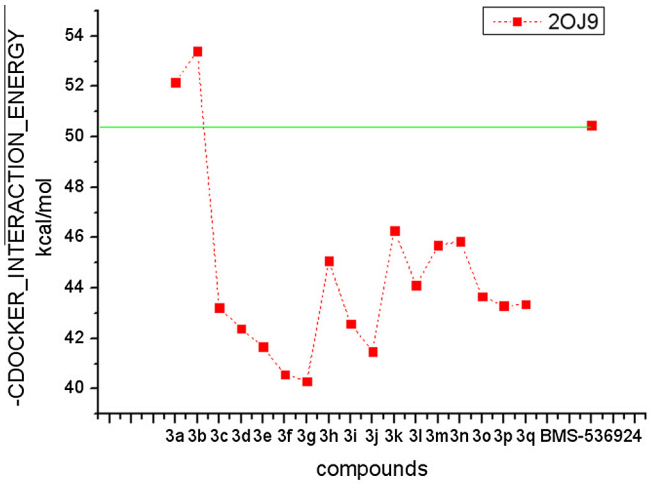
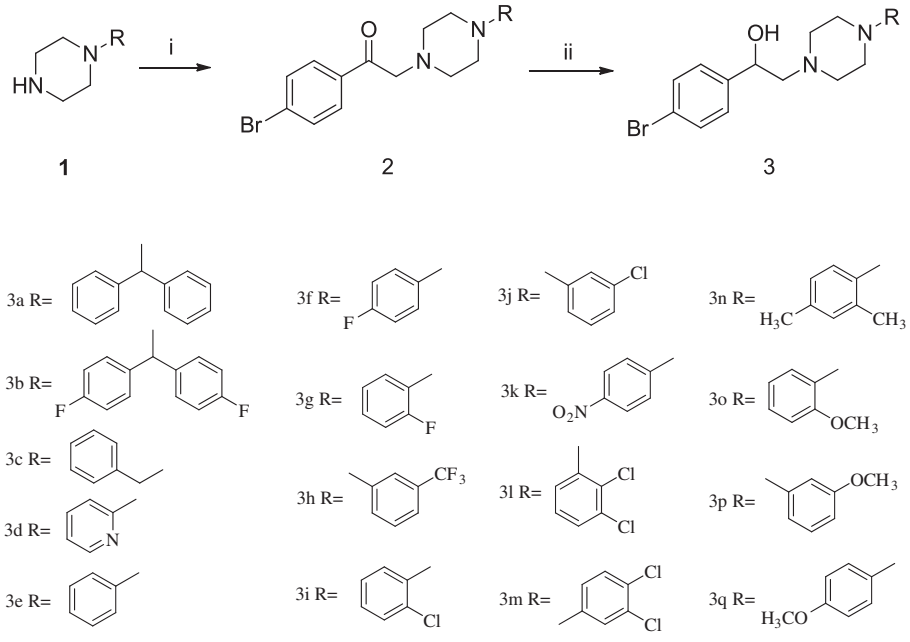


Figure 2. The CDOCKER_INTERACTION_ENERGY (kcal/mol) obtained from the docking study of all synthesized compounds by the CDOCKER protocol (Discovery Studio 3.1, Accelrys, Co. Ltd).

To continue our study of anticancer drugs,[19] seventeen phenylpiperazine derivatives were synthesized for the first time. The synthetic route of compounds **3a**–**3q** was followed the general pathway outlined in Scheme 1. They are prepared in two steps. Firstly, the intermediates obtained by condensation reaction between the 2-bromo-1-(4-bromophenyl)ethanone and the substituted phenylpiperazine, adding 1.5 times of K_2CO_3 in acetonitrile. Secondly, reduction of intermediates with $NaBH_4$ in ethanol leads to the formation of the final phenylpiperazine derivatives.

All of the synthetic compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures. Additionally, the structure of compound **3p** was further confirmed by X-ray diffraction. The CCDC deposition number of **3p** is 1039756. Its crystal data and hydrogen bond date are presented in Tables 1 and 2, respectively, and Figure 3 gives a perspective view of this compound together with the atomic labeling system.

To test the anticancer activities of the synthesized compounds, the target compounds were evaluated in vitro antiproliferation assays against four human cancer cell lines MCF-7, LS-741T, SMMC-7721 and SGC-7901 cell lines. The results were summarized in Table 3. With few exception, the active analogs showed a remarkable potential antitumor activity, suggesting that the new compounds could significantly enhance anticancer potency. For the given compounds, it was observed that compound **3b** showed the most potent biological activity (IC_{50} = 0.024 μM for MCF-7, IC_{50} = 0.068 μM for LS-741T, IC_{50} = 0.150 μM for SMMC-7721 and IC_{50} = 0.116 μM for SGC-7901).



Scheme 1. General synthesis of compounds **3a**–**3q**. Reagents and conditions: (i) 2-bromo-1-(4-bromophenyl)ethanone, 1.5 times of K_2CO_3, CH_3CN, reflux; (ii) 1.5 times of $NaBH_4$, CH_3CH_2OH, rt.

Table 1
Crystallographic data, details of data collection and structure refinement parameters

Compound	**3p**
Empirical formula	$C_{19}H_{23}BrN_2O_2$
Formula weight	391.29
Crystal system	Monoclinic
Space group	P21/n
a (Å)	9.8057(6)
b (Å)	20.2577(12)
c (Å)	10.0973(6)
α (°)	90
β (°)	113.182(2)
γ (°)	90
V (Å)	1843.79(19)
Z	4
D calc/g cm^{-3}	1.410
θ range (°)	2.0–25.1
F(000)	808
Reflections collected/unique	17426/3268
Data/restraints/parameters	2029/0/219
Absorption coefficient (mm^{-1})	2.243
$R_1/_wR_2$ [$I > 2\sigma(I)$]	0.0475/0.0960
$R_1/_wR_2$ (all date)	0.0929/0.1107
GOOF	1.013

Table 2
Hydrogen bond lengths (Å) and bond angles (°) of compound **3p**

H-bond type	D–H…A	d(D–H)	d(H…A)	d(D…A)	∠DHA
intra	O3–H3…N1	0.82	2.18	2.678(4)	119
	C2–H2…O3	0.93	2.44	2.763(5)	101
inter	C20–H20A…O3	0.96	2.58	3.524(6)	168
	C20–H20B…O1	0.96	2.59	3.477(5)	153

According to the data presented in Table 3, we could arrive at the conclusion that the activity of the tested compounds may be correlated to the variation and modifications of structure. Structure–activity relationships in these new compounds demonstrated that when the compounds (**3a** and **3b**) were substituted by two phenyl ring, they showed the most potent antiproliferative activity to four tumor cell lines, with IC_{50} concentration range of 0.024–0.253 μM. It was concluded that compounds **3b** and **3a** may be have broad-spectrum antitumor activity against the mentioned four cancer cell lines. The compounds that introduction of two substituent on the phenyl ring (**3n**, **3m**, **3l**) has much better antiproliferative activity than the compounds of single-substituent on the phenyl ring, while cell-based activity is maintained relative to the compound that phenyl ring substituent containing-CF_3 (**3h**). Generally speaking, when the compounds were single-substituent on the substituted phenyl ring, the potency order was $-NO_2 > -OCH_3 > -Cl > -F$, and the antiproliferative activities that having $-NO_2$ phenyl ring substituent (**3k**) are much better than the other derivatives, even considerable with the positive control (BMS-536924). When the compounds were benzyl, pyridyl and phenyl-substituent derivatives (**3c**, **3d**, **3e**), their antiproliferative activities were not remarkable compared with the single-substituent phenyl ring derivatives, and they have one or two orders of magnitude difference with compounds **3a** and **3b**.

In order to verify whether it has damage to normal cell, we tested the cytotoxicity of some new compounds to human hepatocytes QSG7701. The result showed that CC_{50} of all the tested compounds were larger than 200 μM, it means that when the low concentrations new compounds act on human hepatocytes QSG7701 in vitro, the normal cells almost no destruction. The data of cytotoxicity assay and CC_{50} were presented in Table 4.

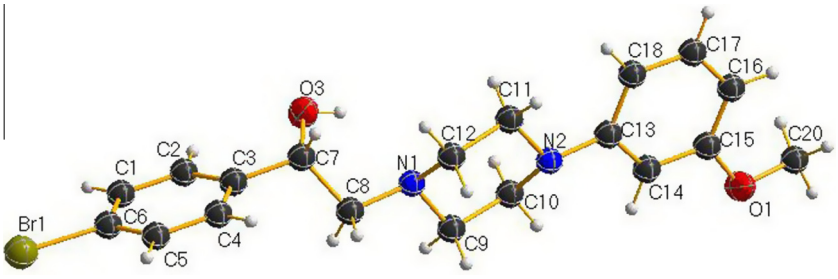


Figure 3. Molecular structure of compound **3p** with atomic numbering scheme.

Table 3
In vitro anticancer activities (IC_{50}, μM) against four human tumor cell lines and IGF-1R inhibitory activities (IC_{50}, μM)

Compounds	IGF-1R (μM) ± SD	IC_{50} (μM) ± SD			
		MCF-7	LS-741T	SMMC-7721	SGC-7901
3a	0.034 ± 0.009	0.037 ± 0.011	0.111 ± 0.009	0.135 ± 0.026	0.253 ± 0.043
3b	0.030 ± 0.009	0.024 ± 0.013	0.068 ± 0.015	0.150 ± 0.029	0.116 ± 0.034
3c	0.568 ± 0.034	2.877 ± 0.024	2.687 ± 0.020	3.535 ± 0.030	3.756 ± 0.037
3d	1.186 ± 0.037	3.410 ± 0.040	6.612 ± 0.049	6.117 ± 0.027	5.696 ± 0.042
3e	1.308 ± 0.035	5.708 ± 0.019	8.991 ± 0.008	7.640 ± 0.025	9.277 ± 0.038
3f	2.081 ± 0.034	11.48 ± 0.035	4.613 ± 0.060	8.157 ± 0.029	10.33 ± 0.031
3g	2.471 ± 0.038	16.36 ± 0.029	5.343 ± 0.046	9.633 ± 0.026	15.95 ± 0.042
3h	0.235 ± 0.039	0.860 ± 0.040	0.963 ± 0.079	1.198 ± 0.033	2.079 ± 0.040
3i	1.107 ± 0.040	2.461 ± 0.027	2.538 ± 0.022	3.918 ± 0.029	4.617 ± 0.041
3j	1.641 ± 0.032	10.38 ± 0.042	16.90 ± 0.031	7.832 ± 0.027	22.29 ± 0.037
3k	0.098 ± 0.036	1.308 ± 0.031	2.181 ± 0.018	1.526 ± 0.029	1.837 ± 0.038
3l	0.265 ± 0.033	1.065 ± 0.034	1.922 ± 0.022	2.035 ± 0.031	2.470 ± 0.038
3m	0.207 ± 0.041	0.777 ± 0.033	1.889 ± 0.025	1.678 ± 0.027	2.141 ± 0.035
3n	0.129 ± 0.040	0.863 ± 0.036	1.824 ± 0.024	1.476 ± 0.029	1.385 ± 0.044
3o	0.270 ± 0.047	1.216 ± 0.049	2.566 ± 0.019	2.277 ± 0.029	4.150 ± 0.041
3p	0.395 ± 0.038	1.444 ± 0.034	2.062 ± 0.024	2.906 ± 0.032	3.285 ± 0.042
3q	0.374 ± 0.033	1.339 ± 0.059	2.598 ± 0.007	2.824 ± 0.044	3.464 ± 0.039
BMS-536924	0.153 ± 0.047	1.748 ± 0.029	2.050 ± 0.034	2.555 ± 0.031	2.893 ± 0.042

Please cite this article in press as: Guo, F.-J.; et al. *Bioorg. Med. Chem. Lett.* (2015), http://dx.doi.org/10.1016/j.bmcl.2015.01.011

Table 4
In vitro cytotoxicity activities (CC_{50}, μM) against human hepatocytes QSG7701

Compounds	3a	3e	3h	3k	3m	BMS-536924
CC_{50} (μM)	2.0×10^3	2.5×10^2	6.6×10^3	1.6×10^3	5.6×10^5	3.2×10^4

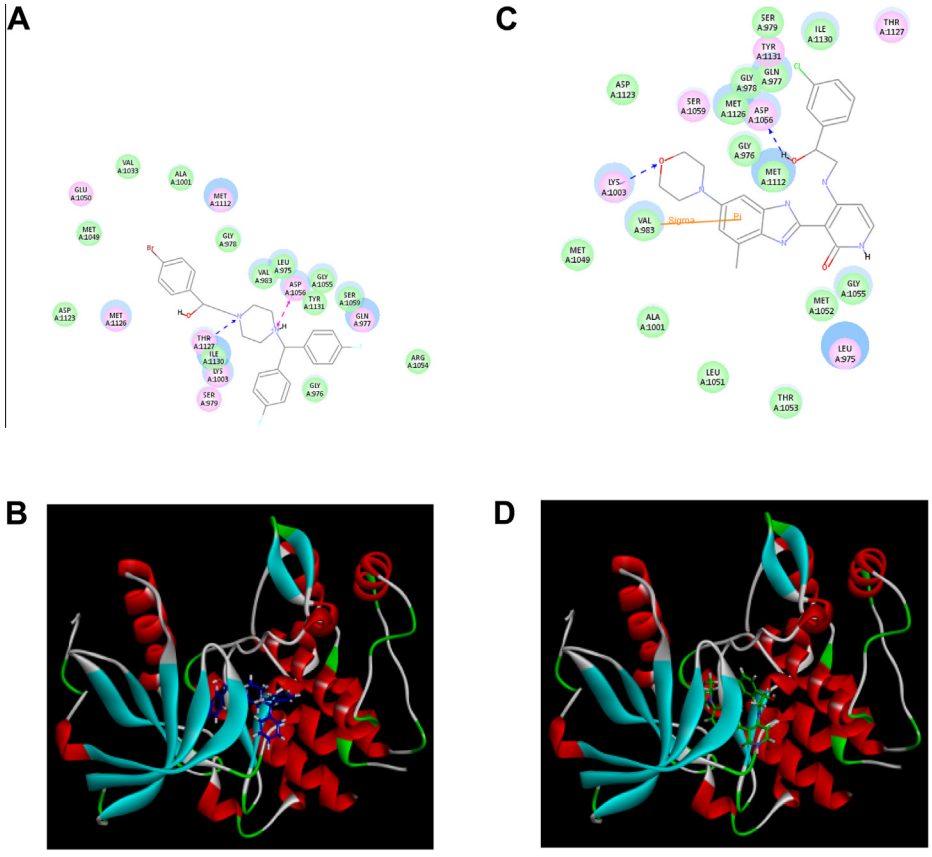


Figure 4. (A) 2D molecular docking model of compound **3b** with 2OJ9. (B) 3D interaction map between compound **3b** and 2OJ9 binding site. (C) 2D diagram of docking structure of compound BMS-536924 with 2OJ9. (D) 3D Model of the interaction between compound BMS-536924 and 2OJ9 binding site.

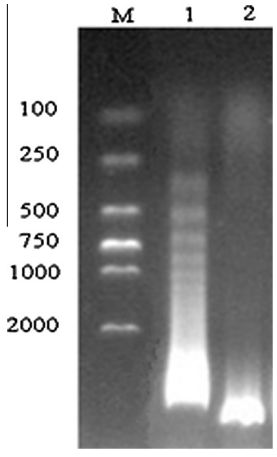


Figure 5. The result of apoptosis induced assay of compound **3b** to MCF-7 cancer cell line. The first strip named M means the DL 2000 DNA Marker. The second strip named 1 is the DNA Ladder that caused by compound **3b** in the concentration of IC_{50}. The third strip named 2 is the negative control which without any drug treatment to the normal cell.

To validate whether the above anti-proliferative effect was produced by interaction of IGF-1R protein and the synthesized compounds, the synthesized compounds were evaluated for their abilities to inhibit the activity of IGF-1R relevant to cancer. As expected, all compounds displayed the potent inhibitory activity for IGF-1R and the results were showed in Table 3. Among them, compound **3b** showed the most potent inhibitory with IC_{50} of 0.025 μM, and sixteen-fold improvement in enzymatic potency than the positive control BMS-536924. The results of IGF-1R inhibitory activity of the tested compounds were in agreement to the structure relationships (SAR) of their antiproliferative activities. This agreement suggested that antiproliferative activities of the synthesized compounds would derive from the inhibition of IGF-1R enzymatic activities.

To help understand the new compounds observed at the IGF-1R and guide further phenylpiperazine studies, molecular docking of the most potent inhibitor **3b** into active binding site of IGF-1R was performed on the binding model based on the IGF-1R complex structure (PDB code: 2OJ9). The obtained results were presented in Figure 4. Figure 4A and B showed the binding mode of compound **3b** interacting with IGF-1R protein, the docking results revealed that two amino acids Asp1056 and THR1127 located in the binding pocket of protein played a vital roles in the conformation with compound **3b**, which were stabilized by two hydrogen bonds. Figure 4C and D displayed 2D and 3D diagram of docking structure of compound BMS-536924 with 2OJ9 binding site. Insight into those two pictures, we can see that amino acid residue Asp1056 located in the binding pocket also seemed very important for the active

conformation of the positive control. These results could provide a molecular level foundation to illustrate compound **3b** can bind well at the active site of IGF-1R tyrosine kinase.

We evaluated compound **3b** for its ability to induce apoptosis in the MCF-7 cell line used cellular DNA extraction kit to extract genomic DNA. The results were shown in Figure 5. As can be seen, compound **3b** is very effective in induction the apoptosis of MCF-7 cell. This is consistent with its nice binding affinity to IGF-1R TK and its potent activity in inhibition of cell growth.

In summary, novel phenylpiperazine analogues were designed and synthesized. Structure-activity relationships were investigated by introducing different substituent into the piperazine ring. Several compounds showed excellent antiproliferative activity which were comparable to existing IGF-1R-targeting agents, such as BMS-536924. Compound **3b** demonstrated the most potent inhibitory activity that inhibited the activity of IGF-1R with IC_{50} of 0.03 μM. Docking simulation was performed to position compound **3b** into the IGF-1R active site to determine the probable binding conformation and the result indicated that compound **3b** was a potent inhibitor of IGF-1R. Apoptosis assay result showed the compound **3b** can induce the programmed cell death of MCF-7 cell line. These results strongly suggest that novel phenylpiperazine analogues can be further developed as a promising antitumor agent for the more efficacious treatment of advanced cancers.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.01.011.

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