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Citation for final published version:

Elseginy, Samia A., Lazaro, Glorianne, Nawwar, Galal A. M., Amin, Kamilia M., Hiscox, Stephen E. and Brancale, Andrea 2015. Computer-aided identification of novel anticancer compounds with a possible dual HER1/HER2 inhibition mechanism. *Bioorganic and Medicinal Chemistry Letters* 25 (4) , pp. 758-762. 10.1016/j.bmcl.2014.12.095 file

Publishers page: <http://dx.doi.org/10.1016/j.bmcl.2014.12.095>  
<<http://dx.doi.org/10.1016/j.bmcl.2014.12.095>>

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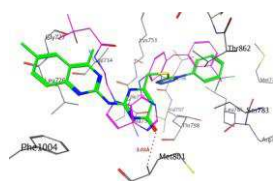
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### **Computer-Aided Identification of Novel Anticancer Compounds with a Possible Dual HER1/HER2 Inhibition Mechanism.**

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# Computer-Aided Identification of Novel Anticancer Compounds with a Possible Dual HER1/HER2 Inhibition Mechanism.

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## ARTICLE INFO

### Article history:

Received

Revised

Accepted

Available online

### Keywords:

HER1 EGFR

HER2

Virtual Screening Structure-based drug design

## ABSTRACT

HER1 and HER2 are frequently overexpressed in human tumors where they drive cellular proliferation. For this reason they are considered important targets in anticancer therapy with dual HER1/HER2 inhibitors being recently approved and marketed. In this paper we report the identification of a series of compounds with anticancer activity by a combined virtual screening approach on the kinase domains of HER1 and HER2. 6 Hit compounds that present a sub- or low- micromolar activity in two cell-based assays, were initially identified and a subsequent design cycle led to the synthesis of a compound with nanomolar activity in the cell-based assays.

The ErbB receptor family are multidomain proteins that contain an extracellular ligand binding domain, a single transmembrane domain, and an intracellular tyrosine kinase domain.<sup>1,2</sup> This family consists of EGFR (HER1, ErbB1), HER2 (ErbB2, HER2/neu), HER3 (ErbB3), and HER4 (ErbB4). The receptors play a role in the regulation of cell proliferation, differentiation, and migration.<sup>3</sup> Upon ligand binding to the extracellular domain, the receptors can form either homo- or hetero-dimers through which the cytoplasmic domains become catalytically active and undergo C terminus phosphorylation, further initiating downstream signalling events.<sup>4,5</sup> HER2 appears to be the preferred binding partner of EGFR and the HER2/EGFR heterodimer shows an increased signalling potency relative to EGFR homodimers.<sup>6</sup> HER2 itself also undergoes spontaneous homo- or heterodimerization and activates downstream signalling.<sup>7</sup> HER2 is overexpressed in a number of human cancers, including 20–40% of solid tumours including breast, ovarian, lung, gastric, and oral cancers where it correlates with poor prognosis.<sup>8–10</sup> Given that HER2 is only expressed at low levels in normal human tissues, its overexpression in tumours makes it an attractive target for tumour-specific therapies.

Two approaches have been developed for blocking the upregulated HER2 signal pathway. One approach utilizes the anti-HER2 monoclonal antibody, trastuzumab.<sup>11–13</sup> This blocks the extracellular ligand-binding region of the receptor, thereby interfering with its activation and modulating HER2-dependent signalling. An alternative approach for blocking upregulated HER2 signal pathways involves the use of orally active small molecule tyrosine kinase inhibitors (TKIs) several of which have been developed. Among these, CP-724714 has been reported as a HER2-specific TKI.<sup>14–15</sup> As many types of malignancies are characterized by overexpression of both HER2 and EGFR, some

interest has been focused on the development of dual inhibitors. Lapatinib, a dual TKI for HER2 and EGFR, was approved in 2007 in combination with capecitabine or letrozole in patients with metastatic breast cancer that overexpress the HER2 receptor.<sup>16,17</sup> Irreversible inhibitors - Neratinib/HKI-272<sup>18,19</sup> and Afatinib/BIBW-2992<sup>20–21</sup> - and reversible inhibitors - AEE-788<sup>22</sup> and BMS-599626<sup>23</sup> - have also been tested in clinical trials. In addition to breast cancer, these HER2/EGFR dual kinase inhibitors are being investigated in a number of solid tumours including lung, gastric, and prostate cancers.<sup>16–23</sup>

In the present study we report the computer-aided identification and biological evaluation of a series of novel compounds which binds to the kinase domains of both HER2 and EGFR..

Our initial step was to perform a virtual screening against the two kinase domains of the target proteins (HER1 and HER2), using the SPECS library (~200,000 compounds).<sup>24</sup> The three-dimensional structures of HER2 (PDB code: 3RCD)<sup>25</sup> and HER1 (PDB code: 3POZ)<sup>26</sup> used in the molecular modelling simulations were prepared using the protonate-3D function in MOE.<sup>27</sup> The ligand library was also prepared with MOE, using the import conformations option, with the default settings. Initially, the compound library was filtered through a pharmacophore query, which was created by using the interactions of the co-crystallised ligand with the HER2 kinase domain (TAK-285, a dual kinase inhibitor). Filtering in this manner, resulted in 13017 hit structures, which were then docked using PLANTS.<sup>28</sup> The results obtained, were then rescored using two other molecular docking software: Glide (Standard Precision)<sup>29</sup> and LeadIT.<sup>30</sup> A simple consensus score between the three scoring function was then used to rank the results.<sup>31</sup> The top 848 compounds were then docked again using the extra precision

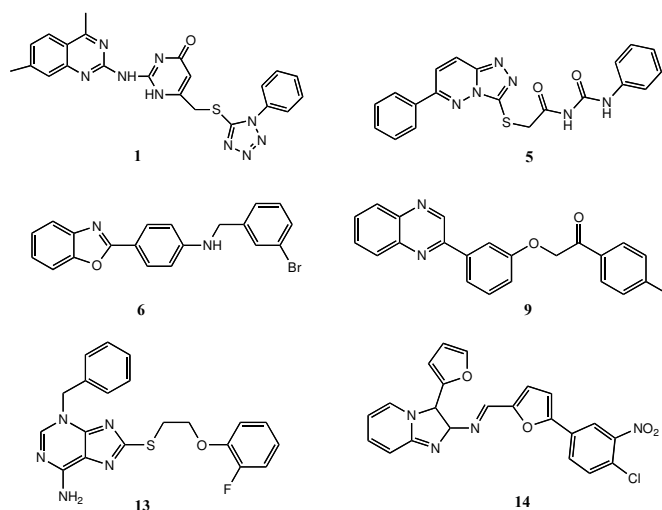
(XP) mode and the refining option in Glide. At this point, the top 472 structures obtained in the described simulation, were docked in the EGFR kinase domain structure, using the same docking/scoring approach described for above, but without using the initial pharmacophore-based filter. The docking poses of the top 200 structures were then visually inspected to remove all the poses that did not completely occupy the binding pocket of both structures. Finally, the 15 top-scored compounds that showed a consistent binding mode in the two kinases, were selected for biological evaluation.

**Table 1.** Effects of the virtual screening hits 1 on BT474 cell growth at a 10 $\mu$ M concentration.

Compound	SPECS ID	% inhibition
1	AJ-292/42284615	72.74 $\pm$ 0.672
2	AG-690/09179059	39.78 $\pm$ 1.69
3	AK-778/43114822	7.255 $\pm$ 1.14
4	AO-476/40829154	48.19 $\pm$ 9.24
5	AO-476/43415760	52.66 $\pm$ 4.82
6	AN-465/43369940	51.76 $\pm$ 6.7
7	AN-465/43411129	27.2 $\pm$ 3.36
8	AP-263/43370995	16.99 $\pm$ 4.21
9	AK-918/42179447	56.25 $\pm$ 5.76
10	AN-465/42834516	50.96 $\pm$ 4.3
11	AN-979/15447113	35 $\pm$ 4.6
12	AO-476/43380211	9.56 $\pm$ 2.6
13	AG-670/40909957	61.37 $\pm$ 4.07
14	AG-690/36158020	76.99 $\pm$ 6.07
15	AN-329/42285780	47.923 $\pm$ 6.90
Herceptin		40.19 $\pm$ 0.86

Initially, we assessed the selected molecules against the BT474 breast cancer cell line (Table 1), which overexpresses HER2 and oestrogen receptors.<sup>32</sup> The compounds were screened at a 10 $\mu$ M concentration in a colorimetric MTT cell proliferation assay to assess changes in cell growth.<sup>33</sup> All compounds were assessed against a control consisting of cell culture media alone containing the appropriate amount of drug solvent (DMSO).

A more accurate IC<sub>50</sub> was then calculated for the 6 compounds that showed the highest percentage of growth inhibition in this assay (Figure 1). These compounds were also evaluated against the HER2+/oestrogen receptor-negative SKBR3 cell line (Table 2). The results showed that compound **1** has best IC<sub>50</sub> against SKBR3 cell line (1.03 $\pm$ 0.413 $\mu$ M) whereas the best IC<sub>50</sub> against BT474 was for compound **9** (0.3139  $\pm$  0.1069  $\mu$ M).



**Figure 1.** Structures of the compounds that have shown activity in the first assay against the BT474 cell line.

**Table 2.** Calculated IC<sub>50</sub> against BT474 and SKBR3 cell lines for selected compounds.

Compound	BT474 ( $\mu$ M)	SKBR3 ( $\mu$ M)
1	0.755 $\pm$ 0.1126	1.03 $\pm$ 0.413
5	0.8023 $\pm$ 0.145	1.64 $\pm$ 0.062
6	0.343 $\pm$ 0.063	1.24 $\pm$ 0.66
9	0.3139 $\pm$ 0.1069	3.11 $\pm$ 0.415
13	0.4261 $\pm$ 0.083	2.26 $\pm$ 0.672
14	1.097 $\pm$ 0.1684	3.17 $\pm$ 0.470
Herceptin	1.398 $\pm$ 0.452	1.07 $\pm$ 0.360

To have an indication that these compounds bind to the intracellular kinase domains of HER1 and HER2, we performed an ELISA assay for both proteins.<sup>34</sup> This specific assay indicates the ability of the tested compounds to inhibit the binding of an antibody that specifically recognises the intracellular C-terminal domains (the kinase domain) of the two proteins.<sup>35</sup> This inhibition could occur as a direct competition effect between the ligand and the antibody or by the induction of a conformational change in the kinase domain caused by the bound ligand that alters the antibody recognition site. In both scenarios, the assay would indicate if the reported compounds bind to the kinase domain of the receptors. We carried out the ELISA assays on the 6 hits (table 3). All these molecules were tested at a concentration of 10 $\mu$ M and all showed a >67% of binding inhibition. In particular, compound **1** showed a >80% inhibition against both HER1 and for HER2, with calculated IC<sub>50</sub> of 3.92 $\mu$ M for HER1 and 6.029 $\mu$ M for HER2.

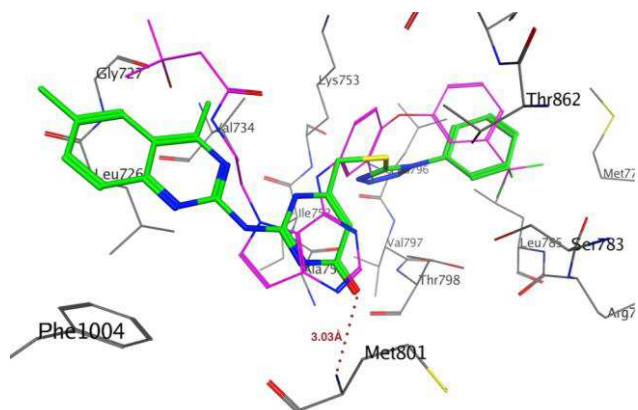
**Table 3.** ELISA assay results of selected compounds against HER2/HER1, as percentage of binding inhibition at 10 $\mu$ M ligand concentration

Compound	HER1	HER2
1	83.02 $\pm$ 3.635	85.75 $\pm$ 0.288
5	76.063 $\pm$ 1.955	75.06 $\pm$ 0.106
6	79.89 $\pm$ 1.053	73.14 $\pm$ 0.123
9	83.900 $\pm$ 6.5	67.04 $\pm$ 0.13
13	81.80 $\pm$ 2.8	72.10 $\pm$ 0.3
14	80.53 $\pm$ 0.7	67.09 $\pm$ 0.116

The proposed binding configuration of compound **1** in the active site of the HER2 kinase domain is shown in Figure 2. Compound **1** appears to form a H-bond with the Met801 NH and a series of hydrophobic interactions with Leu852, Leu762, Phe1004, Thr798, Thr862 and Leu785. The compound has also a very similar binding in HER1 kinase domain, forming H-bonds with the Met793 NH and the Lys745 side chain amino group, besides hydrophobic interaction with Leu718 (not shown).

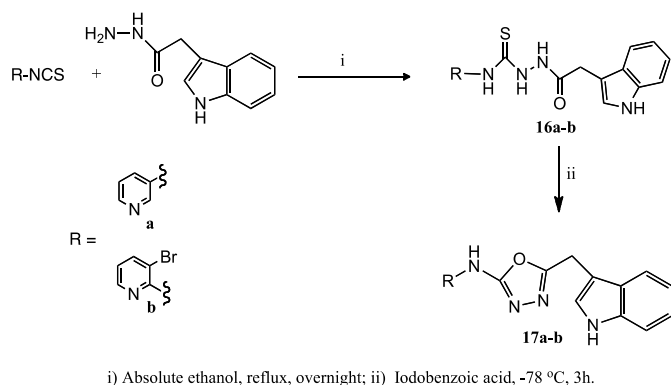
Indeed, there appeared to be some commonality in the binding of the 6 hit compounds (e.g. interaction with the corresponding Met in both HER1 and HER2) and, based on the poses obtained from the docking simulations, we decided to merge a set of structural features of the different hits in a single scaffold. In

particular, we designed a novel series of compound that present an indole ring as a simplified bicyclic heterocycle (a group present in all the hit structures) and a tiosemicarbazide as a replacement of the urea group present in **5**. Furthermore, the tiosemicarbazide could be easily cyclised to generate an oxadiazole ring, which could mimic the furan ring of **14**. Based on these concepts, we docked a series of novel structures (both onto HER1 and HER2) to identify the most promising compounds to be prepared and biologically evaluated. The docking results obtained for compound **16b** is shown in Figure 3 (HER1).



**Figure 2.** Docking pose of **1** (represented in green) in the binding pocket of HER2. Co-crystallised ligand (TAK-285) is represented in purple.

Synthesis of **17a-b** was achieved by condensation of the commercially available indoleacetic acid hydrazide with the appropriate isocyanate derivatives in absolute ethanol. The reaction produced a precipitate that was then collected and recrystallized from ethanol to yield **16a-b**. The oxadiazole derivatives **17a-b** were then prepared by cyclisation of **16a-b** in presence of iodobenzoic acid in basic condition at  $-78^{\circ}\text{C}$  for 3hr (Scheme 1).<sup>36</sup>



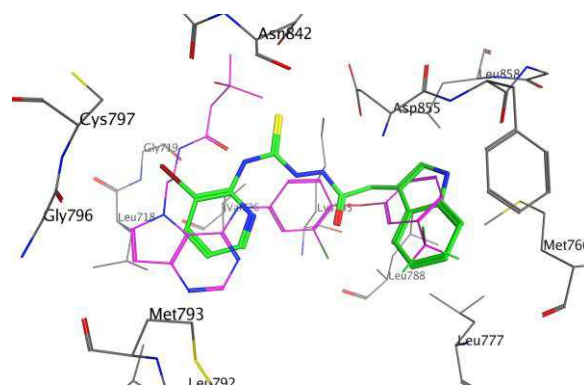
**Scheme 1.** Preparation of **16a-b** and **17a-b**.

The evaluation of newly synthesised compounds was carried initially against the SKBR3 cell line at a concentration of  $10\mu\text{M}$  (Table 4).

**Table 4.** Effects of the synthesized compounds on SKBR3 cell growth at a  $10\mu\text{M}$  concentration.

Compound	% inhibition
<b>16a</b>	21.245±2.21
<b>16b</b>	71.51±3.32
<b>17a</b>	33.36±1.32
<b>17b</b>	37.17±4.10

Compound **16b** showed the best inhibition (71.51%) in the series and a more accurate biological evaluation was then performed resulting in a  $\text{IC}_{50}$  value of  $0.01\pm 0.0028\mu\text{M}$  against SKBR3 and  $0.055\pm 0.0212\mu\text{M}$  against BT474. A further evaluation was done using the ELISA assay, described above, against HER1 and HER2 proteins. This assay confirmed the binding of compound **16b** to the kinase domain of HER2 ( $\text{IC}_{50}$   $12.37\pm 2.50\mu\text{M}$ ), and binding to HER1 ( $\text{IC}_{50}$  of  $8.49\pm 2.20\mu\text{M}$ ). It should be noted that, for this compound, the cell-based activity is considerably lower than the one observed in the binding assay. However, as the ELISA assays provide an indication of the binding of the compound to the kinases, and they do not assess directly enzymatic inhibition, a strict correlation between the results of the biochemical and cell-based anticancer assay might not be necessarily observed. Furthermore, at this point, we cannot exclude the involvement of other cellular targets beside HER1 and HER2.



**Figure 3.** Docking pose of **16b** (represented in green) in the binding pocket of HER1. Co-crystallised ligand (TAK-285) is represented in purple.

In conclusion, we reported the discovery, using a virtual screening approach, of a series of novel compounds that shows potent anticancer activity against both the BT474 and SKBR3 cell lines. Furthermore, binding of these compounds to the kinase domains of HER1 and HER2 was assessed using an ELISA assay. Among these structures, compound **1** showed the lowest  $\text{IC}_{50}$  ( $1.03\pm 0.413\mu\text{M}$ ) against the SKBR3 cell line. Furthermore, a novel series of compounds was then designed using the structural information of the hit molecules obtained from the *in silico* screening. These novel structures were then biologically evaluated and compound **16b** showed nanomolar activity in both cell-based assays. Furthermore, the ELISA assay suggests that **16b** could target HER1 and HER2 kinase domains. However, further studies will be needed to confirm the mode of action for these compounds. We believe that both **1** and **16b** represent a good starting point for the development of novel anticancer compounds, with a possible HER1/HER2 dual inhibition mechanism of action.

## Acknowledgments

The authors are grateful to the Higher Education Ministry of the Arab Republic of Egypt for the award of a PhD Channel Scholarship (to S.A.). The authors are also very grateful to Dr. Marcella Bassetto for her kind help and support.

## References and notes

- Carter, P.; Presta, L.; Gorman, C. M.; Ridgway, J. B.; Henner, D.; Wong, W. L.; Rowland, A. M.; Kotts, C.; Carver, M. E.; Shepard, H. M. *Proc. Natl. Acad. Sci. U.S.A.*, **1992** *89*, 4285.

2. Slamon, D. J.; Godolphin, W.; Jones, L. A.; Holt, J. A.; Wong, S. G.; Keith, D. E.; Levin, W. J.; Stuart, S. G.; Udove, J.; Ullrich, A.; Press, M. F. *Science*, **1989**, *244*, 707.
3. Schlessinger, J. *Science*, **2004**, *306*, 1506.
4. Yarden, Y.; Sliwkowski, M. X. *Nat. Rev. Mol. Cell Biol.* **2001**, *2*, 127.
5. Qian, X.; LeVea, C. M.; Freeman, J. K.; Dougall, W. C.; Greene, M. I. *Proc. Natl. Acad. Sci. U.S.A.*, **1994**, *91*, 1500.
6. Lenferink, A. E.; Pinkas-Kramarski, R.; Van de Poll, M. L.; Van Vugt, M. J.; Klapper, L. N.; Tzahar, E.; Waterman, H.; Sela, M.; Van Zoelen, E. J.; Yarden, Y. *EMBO J.*, **1998**, *17*, 3385.
7. Hynes, N. E.; Stern, D. F. *Biochim. Biophys. Acta*, **1994**, *1198*, 165.
8. Berchuck, A.; Kamel, A.; Whitaker, R.; Kerns, B.; Olt, G.; Kinney, R.; Soper, J. T.; Dodge, R.; Clarke-Pearson, D. L.; Marks, P.; McKenzie, S.; Yin, S.; Bast, R. C. Jr. *Cancer Res.*, **1990**, *50*, 4087.
9. Slamon, D. J.; Clark, G. M.; Wong, S. G.; Levin, W. J.; Ullrich, A.; McGuire, W. L. *Science*, **1987**, *235*, 177.
10. Gschwind, A.; Fischer, O. M.; Ullrich, A. *Nature Rev. Cancer*, **2004**, *4*, 361.
11. Baselga, J.; Norton, L.; Albanell, J.; Kim, Y.; Mendelsohn, J. *Cancer Res.*, **1998**, *58*, 2825.
12. Molina, M. A.; Codony-Servat, J.; Albanell, J.; Rojo, F.; Arribas, J.; Baselga, J. *Cancer Res.*, **2001**, *61*, 4744.
13. Gennari, R.; Menard, S.; Fagnoni, F.; Ponchio, L.; Scelsi, M.; Tagliabue, E.; Castiglioni, F.; Villani, L.; Magalotti, C.; Gibelli, N.; Oliviero, B.; Ballardini, B.; Da Prada, G.; Zambelli, A.; Costa, A. *Clin. Cancer Res.*, **2004**, *10*, 5650.
14. Bhattacharya, S. K.; Cox, E. D.; Kath, J. C.; Mathiowetz, A. M.; Morris, J.; Moyer, J. D.; Pustilnik, L. R.; Rafidi, K.; Richter, D.T.; Su, C.; Wessel, M. D. *Biochem. Biophys. Res. Commun.*, **2003**, *307*, 267.
15. Jani, J. P.; Finn, R. S.; Campbell, M.; Coleman, K. G.; Connell, R. D.; Currier, N.; Emerson, E. O.; Floyd, E.; Harriman, S.; Kath, J. C.; Morris, J.; Moyer, J. D.; Pustilnik, L. R.; Rafidi, K.; Ralston, S.; Rossi, A. M.; Steyn, S. J.; Wagner, L.; Winter, S. M.; Bhattacharya, S. K. *Cancer Res.*, **2007**, *67*, 9887.
16. Rusnak, D. W.; Lackey, K.; Affleck, K.; Wood, E. R.; Alligood, K. J.; Rhodes, N.; Keith, B. R.; Murray, D. M.; Glennon, K.; Knight, W. B.; Mullin, R. J.; Gilmer, T. M. *Mol. Cancer Ther.*, **2001**, *1*, 85.
17. Wood, E. R.; Truesdale, A. T.; McDonald, O. B.; Yuan, D.; Hassell, A.; Dickerson, S. H.; Ellis, B.; Pennisi, C.; Horne, E.; Lackey, K.; Alligood, K. J.; Rusnak, D. W.; Gilmer, T. M.; Shewchuk, L. **2004**, *64*, 6652.
18. Tsou, H. R.; Overbeek-Klumpers, E. G.; Hallett, W. A.; Reich, M. F.; Floyd, M. B.; Johnson, B. D.; Michalak, R. S.; Nilakantan, R.; Discafani, C.; Golas, J.; Rabindran, S. K.; Shen, R.; Shi, X.; Wang, Y. F.; Upeslaci, J.; Wissner, A. *J. Med. Chem.*, **2005**, *48*, 1107.
19. Li, D.; Shimamura, T.; Ji, H.; Chen, L.; Haringsma, H. J.; McNamara, K.; Liang, M.-C.; Perera, S. A.; Zaghlul, S.; Borgman, C. L.; Kubo, S.; Takahashi, M.; Sun, Y.; Chirieac, L. R.; Padera, R. F. *Cancer Cell*, **2007**, *12*, 81.
20. Eskens, F. A. L. M.; Mom, C. H.; Planting, A. S. T.; Gietema, J. A.; Amelsberg, A.; Huisman, H.; van Doorn, L.; Burger, H.; Stopfer, P.; Verweij, J.; de Vries, E. G. *Br. J. Cancer*, **2008**, *98*, 80.
21. Rall, W.; Soyka, R.; Kulinna, C.; Schnaubelt, J.; Sieger, P. International Patent Application WO/2005/037824. **2005**. PCT/EP2004/011378.
22. Yokoi, K.; Thaker, P. H.; Yazici, S.; Rebhun, R. R.; Nam, D. H.; He, J.; Kim, S. J.; Abbruzzese, J. L.; Hamilton, S. R.; Fidler, I. J. *Cancer Res.*, **2005**, *65*, 3716.
23. Wong, T. W.; Lee, F. Y.; Yu, C.; Luo, F. R.; Oppenheimer, S.; Zhang, H.; Smykla, R. A.; Mastalerz, H.; Fink, B. E.; Hunt, J. T.; Gavai, A. V.; Vite, G. D. *Clin. Cancer Res.*, **2006**, *12*, 6186.
24. <http://www.specs.net>
25. Ishikawa T; Seto, M; Banno, H; Kawakita, Y; Oorui, M; Taniguchi T; Ohta, Y; *J. Med. Chem.*, **2011**, *54*, 8030.
26. Aertgeerts K.; Skene R.; Yano J.; Sang B.C.; Zou H.; Snell G.; Jennings A.; Iwamoto K.; Habuka N.; Hirokawa A.; Ishikawa T.; Tanaka T.; Miki H.; Ohta Y.; Sogabe S. *J Biol Chem.*, **2011**, *286*, 18756.
27. Molecular Operating Environment (MOE 2012.10). Chemical Computing Group, Inc. Montreal, Quebec, Canada. [www.chemcomp.com](http://www.chemcomp.com).
28. Korb, O.; Stutzle, T.; Exne, T. E. *J. Chem. Info. Model.*, **2009**, *49*, 84.
29. Glide, version 5.8, Schrödinger, LLC, New York, NY, 2012. <http://www.schrodinger.com>.
30. LeadIt 1.2, BioSolveIT GmbH, Sankt Augustin, Germany, 2012. <http://www.biosolveit.de/>
31. Only the structures that were ranked in the top 25% of each scoring results were considered.
32. Van Slooten H.; Bonsing B.; Hiller A.; Colbern G. *Br. J. Cancer.*, **1995**; *72*, 22.
33. Lazaro, G.; Smith C.; Goddard, L.; Jordan, N.; McCelland R.; Hiscox S *Endocrine-Related Cancer*, **2013**, *20*, 691.
34. Kit purchased from Invitrogen- Thermo Fisher Scientific Inc. <http://www.invitrogen.com> - Cat # KHR9061for HER1 and Cat# HER2 KHO0701.
35. Manual and Protocol provided by the supplier - [http://tools.lifetechnologies.com/content/sfs/manuals/KHR9061\\_R ev1.pdf](http://tools.lifetechnologies.com/content/sfs/manuals/KHR9061_R ev1.pdf)
36. Characterisation of 2-(2-(1H-indole-3-yl)acetyl)-N-(3-bromopyridine-2-yl)hydrazinecarbothioamide (**16b**): Mp 167-169 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>): 3.92 (2H, s, CH<sub>2</sub>), 6.92 (1H, dd, J1 4.99, J2 6.34 ArH(pyridine)), 7.19 (1H, t, J 7.23, ArH (indole)), 7.25-7.29 (2H, m, ArH), 7.43(1H,d, J 8.02,ArH(indole)), 7.65 (1H, d, J 8.02, ArH (indole)), 7.89 (1H, dd,J1 1.51, J2 8.04, ArH(pyridine)), 8.26 (1H, dd, J1 1.4, J2 5.23,ArH (pyridine)), 8.34 (1H, s, NH ) 8.41 (1H, s, NH ), 9.39 (1H, d, J 8.48, NH(hydrazine) ), 14.26 (1H, d, J 7.92 NH(hydrazine); <sup>13</sup>C NMR (CDCl<sub>3</sub>): 31.43 (CH<sub>2</sub>), 106.121 (ArC), 107.14 (ArC), 111.46 (ArCH), 118.65 (ArCH), 119.21 (ArCH), 120.19 (ArCH),122.67 (ArCH),123.71 (ArCH), 126.94 (ArC), 136.35 (ArC), 141.87 (ArCH), 145.19 (ArCH),149.36 (ArC), 166.18 (C=S), 171.42 (C=O); MS (ESI+) 403(M+), 405(M++2); Elemental Anal. Calc. for C<sub>16</sub>H<sub>14</sub>BrN<sub>3</sub>OS: C 47.53, H 3.49, N 17.32, Found: C 47.15, H 3.36, N 16.99.