

Article

# Discovery of *N*-(Naphtho[1,2-*b*]Furan-5-yl) Benzenesulfonamides as Novel Selective Inhibitors of Triple-Negative Breast Cancer (TNBC)

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**Abstract:** Any type of breast cancer not expressing genes of the estrogen receptor (ER), progesterone receptor (PR), or human epidermal growth factor receptor 2 (HER2) is referred to as triple-negative breast cancer (TNBC). Accordingly, TNBCs do not respond to hormonal therapies or medicines targeting the ER, PR, or HER2. Systemic chemotherapy is therefore the only treatment option available today and prognoses remain poor. We report the discovery and characterization of *N*-(naphtho[1,2-*b*]furan-5-yl)benzenesulfonamides as selective inhibitors of TNBCs. These inhibitors were identified by virtual screening and inhibited different TNBC cell lines with IC<sub>50</sub> values of 2–3 μM. The compounds did not inhibit normal (i.e. MCF-7 and MCF-10A) cells in vitro, indicating their selectivity against TNBC cells. Considering the selectivity of these inhibitors for TNBC, these compounds and analogs can serve as a promising starting point for further research on effective TNBC inhibitors.

**Keywords:** triple-negative breast cancer; three-dimensional similarity search; virtual screening; selective inhibitors

## 1. Introduction

Breast cancer is the most common malignancy and second leading cause of cancer death among women in the United States [1]. As in most countries, breast cancer is the most common cancer in Chinese women today. Cases in China account for 12.2% of all newly diagnosed breast cancers and for 9.6% of all deaths from breast cancer worldwide [2]. Based on DNA microarray expression profiling, breast cancers can be classified into six different subtypes [3–8]: luminal A, luminal B, human epidermal growth factor receptor-2 (HER2)-overexpressing, normal breast tissue-like, basal-like, and claudin-low breast cancers. These subtypes respond differently to therapy and are associated with different outcomes, with the shortest survival times seen in patients with basal-like and HER2-overexpressing subtypes [4,5,9].

Triple-negative breast cancer (TNBC) is an aggressive clinical phenotype characterized by the lack of expression (or minimal expression) of the estrogen receptor (ER) and progesterone receptor (PR) as well as the absence of the human epidermal growth factor receptor-2 (HER2). TNBCs comprise a heterogeneous subgroup of tumors, including but not limited to those classified by expression profiling as basal-like and claudin-low subtypes. TNBCs account for about 15% of all breast cancers [7–10]. Unlike patients suffering from ER/PR-positive or HER2-overexpressing cancers, treatment options

for patients with TNBC are currently limited to systemic cytotoxic chemotherapy [11]. The overall survival rates of TNBC patients are lower than those of patients suffering from other phenotypes of breast cancer (in both early and advanced stages) [12,13]. These facts highlight the urgent need for effective medicines for the treatment of TNBCs.

Currently, compounds targeting the vascular endothelial growth factor (VEGF), poly (ADP-ribose) polymerase (PARP), HSP90, and aurora kinase are under investigation in clinical trials as therapeutics for metastatic TNBCs [14]. Herein, we report the computer-guided discovery of *N*-(naphtho[1,2-*b*]furan-5-yl)benzenesulfonamides as effective and selective inhibitors of TNBCs. These compounds serve as starting points for the development of effective drugs.

## 2. Results and Discussion

### 2.1. Three-Dimensional Similarity Search and Bioassays

Estrogens are known to stimulate cell proliferation and increase the risk of the development of several different types of cancers, in particular breast and uterus cancers [15]. In order to identify novel inhibitors of breast cancers, we employed similarity-based computational approaches to search the SPECS compound library (<http://www.specs.net/>, accessed by May 2014) for candidate compounds.  $17\beta$ -estradiol and IC-163 (Figure 1), a potential agent for breast cancer identified by Beijing Shenogen Biomedical Co. [16], were chosen as query molecules for 3D similarity search (Figure 2).

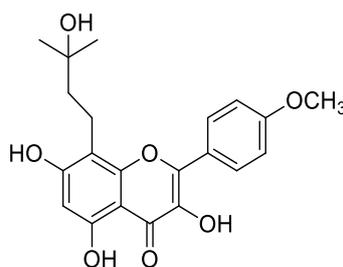
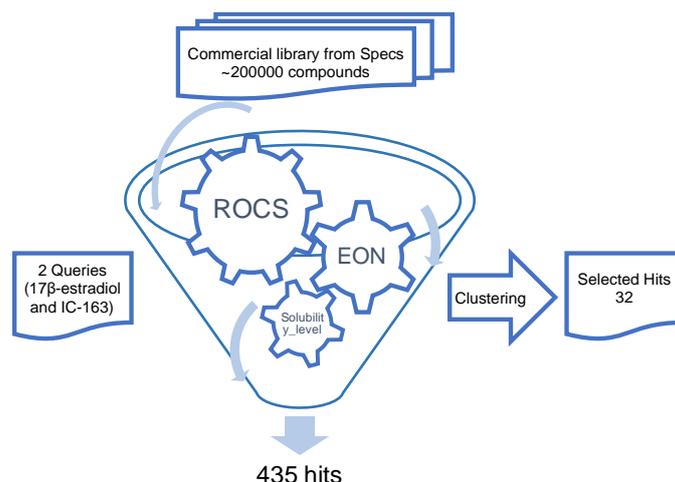


Figure 1. Chemical structure of IC-163.

In total, approximately 200 k compounds were screened with ROCS, an alignment-based virtual screening engine quantifying the similarity of pairs of molecules based on their molecular shapes and chemical features [17,18]. The most interesting compounds (selected by visual inspection) were re-ranked with EON (version 2.2.0, OpenEye Scientific Software Inc., Santa Fe, NM, USA) to evaluate compound similarity with regard to electrostatics. EON quantifies the similarity between pairs of molecules based on their electrostatic potential maps. Comparison with EON resulted in rank-ordered list of 435 candidate molecules, which was further reduced by clustering with ECFP\_6 and FCFP\_6 fingerprints. In total, 32 candidate compounds (Figure S1) were selected by visual inspection (taking into account calculated aqueous solubility) and purchased from SPECS for experimental evaluation. Twenty-five of the selected compounds originate from  $17\beta$ -Estradiol as query and seven from IC-163.

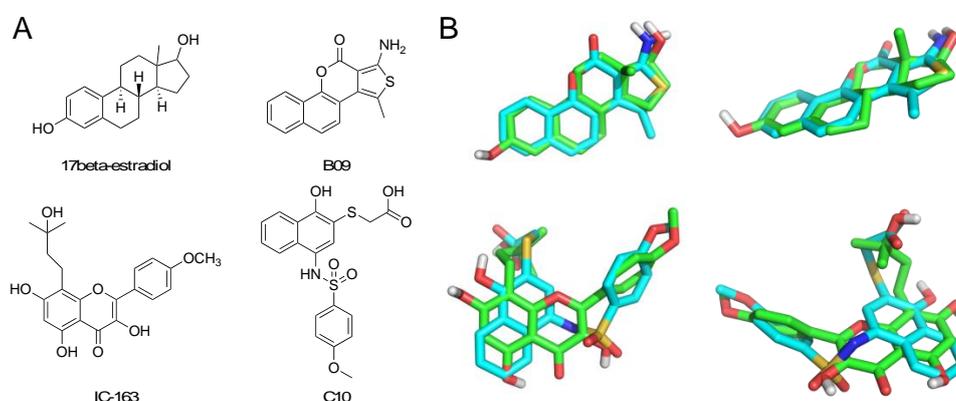


**Figure 2.** Scheme for three-dimensional virtual screening.

The inhibition rates of these 32 compounds were measured on the TNBC cell lines MDA-MB-231 and SUM-159, as well as the non-TNBC breast cancer cell line MCF-7 (Figure S2). The most interesting compounds identified among those 32 candidates were **B09** and **C10**. Compound **B09**, identified by similarity search using  $17\beta$ -estradiol as query, inhibited MCF-7 cells with  $IC_{50} = 1.45 \mu\text{M}$  and had almost no growth-inhibitory effect on MDA-MB-231 and SUM-159.  $17\beta$ -Estradiol is an endogenous molecule directly interacting with the estrogen receptor. This may explain why **B09** only inhibited MCF-7 cells. **B09** is structurally related to  $17\beta$ -estradiol not only with respect to its 3D shape but also its 2D structure (Figure 3). On the contrary, **C10** showed good inhibition of TNBC cell lines ( $IC_{50} = 2.32 \mu\text{M}$  for MDA-MB-231;  $IC_{50} = 3.45 \mu\text{M}$  for SUM-159) but low inhibition of MCF-7 cells ( $IC_{50} = 20 \mu\text{M}$ ; Table 1). Its chemical structure is similar to that of **IC-163** with respect to the 3D molecular shape and electrostatic properties (ShapeTanimoto coefficient = 0.817; EON\_ShapeTanimoto coefficient = 0.784, where values of 1 denote compounds with identical properties) but not with respect to the 2D structure (Figure 3).

**Table 1.** Activities of **B09** and **C10** measured on different cell lines.

Compound	$IC_{50}$ ( $\mu\text{M}$ )		
	MDA-MB-231	SUM-159	MCF-7
B09	~20	>40	1.45
C10	2.32	3.45	20



**Figure 3.** (A) Structures of  $17\beta$ -estradiol, **B09**, **IC-163** and **C10**; (B) Structure alignment of **B09** with  $17\beta$ -estradiol and of **C10** with **IC-163**.

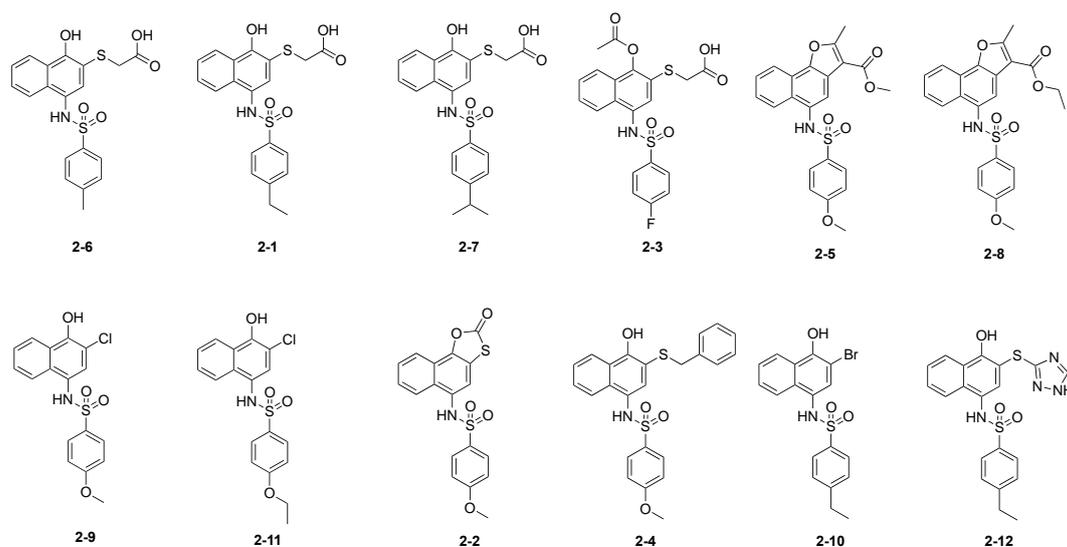
## 2.2. Hit Follow-up and Expansion

2D similarity search based on ECFP<sub>6</sub> and FCFP<sub>6</sub> was conducted to identify further purchasable analogs of **C10** for experimental evaluation. A total of 12 analogs of **C10** were purchased from SPECS and tested on MDA-MB-231 and SUM-159 cell lines. The measured inhibition rates are reported in Figure S3. All compounds were initially tested only with two TNBC cell lines for cell viability at 5 µg/mL. Following this test, the inhibitory activity of any compounds with inhibition rates above 30% at 5 µg/mL (eight compounds) were tested on four TNBC cell lines and one non-TNBC cell line MCF-7 (Table 2). All eight compounds inhibited MDA-MB-231 and MDA-MB-453 cells with IC<sub>50</sub> values lower than 10 µM. Most compounds inhibited SUM-159 and BT-20 cells with IC<sub>50</sub> values greater than 10 µM; seven of these compounds inhibited MCF-7 cells with IC<sub>50</sub> values greater than 40 µM. Among them, Compounds **2-5** and **2-8** exhibited the strongest inhibitory effect on all tested TNBC cell lines and had no inhibitory effect on MCF-7.

**Table 2.** Activities of eight analogs of **C10** measured on different cell lines.

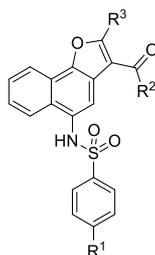
Compound	IC <sub>50</sub> (µM)				
	MDA-MB-231	MDA-MB-453	SUM-159	BT-20	MCF-7
<b>Tamoxifen</b>	2.03	3.64	13.48	8.54	9.08
<b>IC-163</b>	>40	>40	>40	>40	>40
<b>C10</b>	5.34	2.30	11.13	10.63	9.38
<b>2-1</b>	4.36	3.19	30.76	13.34	13.97
<b>2-3</b>	7.99	8.59	>40	>40	>40
<b>2-5</b>	3.12	2.95	2.91	>40	>40
<b>2-6</b>	4.97	3.36	19.21	25.02	>40
<b>2-7</b>	3.95	3.65	>40	>40	>40
<b>2-8</b>	2.96	3.09	2.50	10.10	>40
<b>2-9</b>	6.22	4.61	18.06	>40	>40
<b>2-11</b>	6.92	5.72	>40	>40	>40

All these compounds are based on a *N*-(naphthalen-1-yl)benzenesulfonamide scaffold (Figure 4), with different decorations in *para* position of the benzenesulfonamide and/or the substituent at the naphthalene ring. Compounds **C10**, **2-1**, **2-3**, **2-6**, and **2-7** are known inhibitors of myeloid cell leukemia 1 (Mcl-1) [19]. Compound **2-11** is an antimalarial heme detoxification protein (HDP) inhibitor [20], and **2-9** is an antitumor agent with inhibition of signal transducer and activator of transcription 3 (STAT3) [21,22]. We did not identify literature on the bioactivity of **2-5** and **2-8**. In terms of molecular structure, **2-5** and **2-8** clearly differ from other compounds of that series. They are decorated with a naphtho[1,2-*b*]furan in *para* position of the benzenesulfonamide. Compound **2-5** is a methyl carboxylate, while **2-8** is an ethyl carboxylate. Due to structural novelty and good bioactivity, **2-5** and **2-8** were selected for another iteration of hit expansion based on 2D similarity search.



**Figure 4.** Structures of 12 compounds resulting from a similarity search based for C10.

A total of 40 analogs of 2-5 and 2-8 were purchased from SPECS and tested on MDA-MB-231, SUM-159, and MCF-7 cells (Figure S4). Twenty-four of these analogs did not exhibit activity (any compounds with an inhibition rate above 50% were considered to be active). One compound (3-12) inhibited all three of the cell lines, while 3-9, 3-17, 3-18, 3-20, and 3-37 only had an effect on two TNBC cell lines. Eleven compounds inhibited one TNBC cell line. Compounds with substitutions in *para* position of the benzenesulfonamide tended to be more active (Tables 3 and 4). All tested compounds with  $R^3$  = methyl and  $R^1$  = ethoxy were active against TNBC, regardless of the length of  $R^2$ . Compounds with a large substituent in  $R^2$  tended to be less active on SUM-159 cells. Compounds with  $R^1$  = ethyl were more active on MDA-MB-231 with  $R^2$  = methoxy than  $R^2$  = ethoxy. Compounds with a methoxy substituent in  $R^2$  were inactive on SUM-159. Replacement of the methyl moiety at  $R^3$  by a phenyl ring (e.g., 3-11 to 3-22) or other groups (e.g., 2-8 to 3-28) did not result in substantial changes in activity against the three cell lines, indicating that the substituent at the  $R^3$  position is likely less relevant for TNBC inhibition. Compounds with an isopropyl or chlorine substituent in  $R^1$  showed low activity on MDA-MB-231 and no activity on SUM-159 cells. When (1)  $R^3$  was methyl, (2)  $R^2$  was methoxy or ethoxy and (3)  $R^1$  was methyl, ethyl, methoxy, ethoxy, or fluorine, almost all compounds of this combination had different levels of inhibitory activities on both TNBC cell lines.

**Table 3.** Inhibition rates of analogs of 2-5 and 2-8 identified by 2D similarity search (Part 1).

Compound	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	Inhibition%		
				MDA-MB-231	SUM-159	MCF-7
3-17	-OCH <sub>2</sub> CH <sub>3</sub>	-CH <sub>3</sub>	-CH <sub>3</sub>	68.567	60.956	40.549
3-8	-CH <sub>2</sub> CH <sub>3</sub>	-OH	-CH <sub>3</sub>	-1.131	0.221	-6.706
3-32	-CH(CH <sub>3</sub> ) <sub>2</sub>	-OH	-CH <sub>3</sub>	24.718	-0.964	-7.568
3-1	-Cl	-OH	-CH <sub>3</sub>	31.450	-0.754	-12.178
3-9	-OCH <sub>2</sub> CH <sub>3</sub>	-OCH <sub>3</sub>	-CH <sub>3</sub>	63.008	60.778	24.251
3-37	-OCH <sub>2</sub> CH <sub>3</sub>	-OCH <sub>2</sub> CH <sub>3</sub>	-CH <sub>3</sub>	59.687	66.789	42.503
3-23	-OCH <sub>2</sub> CH <sub>3</sub>	-OCH <sub>2</sub> CH <sub>2</sub> OCH <sub>3</sub>	-CH <sub>3</sub>	62.014	49.127	14.707
3-20	-CH <sub>2</sub> CH <sub>3</sub>	-OCH <sub>3</sub>	-CH <sub>3</sub>	53.486	65.439	31.221
3-11	-CH <sub>2</sub> CH <sub>3</sub>	-OCH <sub>2</sub> CH <sub>3</sub>	-CH <sub>3</sub>	41.115	0.175	13.005
3-22	-CH <sub>2</sub> CH <sub>3</sub>	-OCH <sub>2</sub> CH <sub>3</sub>	-Ph	53.616	-0.556	2.238
3-4	-CH(CH <sub>3</sub> ) <sub>2</sub>	-OCH <sub>3</sub>	-CH <sub>3</sub>	37.770	-0.319	14.222
3-31	-CH(CH <sub>3</sub> ) <sub>2</sub>	-OCH <sub>2</sub> CH <sub>3</sub>	-CH <sub>3</sub>	32.074	-1.330	2.952
3-30	-H	-OCH <sub>3</sub>	-CH <sub>3</sub>	50.872	0.862	29.311
3-14	-CH <sub>3</sub>	-OCH <sub>3</sub>	-CH <sub>3</sub>	62.843	48.214	10.194
2-5	-OCH <sub>3</sub>	-OCH <sub>3</sub>	-CH <sub>3</sub>	31.005	56.793	-
3-10	-F	-OCH <sub>3</sub>	-CH <sub>3</sub>	64.477	35.466	34.480
3-33	-Cl	-OCH <sub>3</sub>	-CH <sub>3</sub>	32.822	-0.507	2.819
3-29	-Br	-OCH <sub>3</sub>	-CH <sub>3</sub>	45.039	1.3243	14.480
3-34	-COOH	-OCH <sub>3</sub>	-CH <sub>3</sub>	10.991	-0.042	2.848
3-26	-H	-OCH <sub>2</sub> Ph	-CH <sub>3</sub>	51.036	1.581	25.556
3-36	-CH <sub>3</sub>	-OCH <sub>2</sub> CH <sub>3</sub>	-CH <sub>3</sub>	16.752	-0.123	-4.385
2-8	-OCH <sub>3</sub>	-OCH <sub>2</sub> CH <sub>3</sub>	-CH <sub>3</sub>	62.936	61.771	-
3-3	-OCH <sub>3</sub>	-OCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	-CH <sub>3</sub>	51.145	42.064	29.745
3-25	-OCH <sub>3</sub>	-OCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	-CH <sub>3</sub>	39.505	61.902	48.321
3-28	-OCH <sub>3</sub>	-OCH <sub>2</sub> CH <sub>3</sub>	-CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	61.911	46.000	33.448
3-12	-OCH <sub>3</sub>	-OCH <sub>3</sub>	-C(CH <sub>3</sub> ) <sub>3</sub>	71.378	90.386	76.319
3-39	-F	-OCH(CH <sub>3</sub> ) <sub>2</sub>	-CH <sub>3</sub>	42.330	-0.193	4.948
3-13	-COOH	-OCH <sub>2</sub> CH <sub>3</sub>	-CH <sub>3</sub>	19.635	-0.635	5.137
3-40	-COOH	-OCH <sub>2</sub> CH <sub>3</sub>	-CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	12.487	0.000	2.525

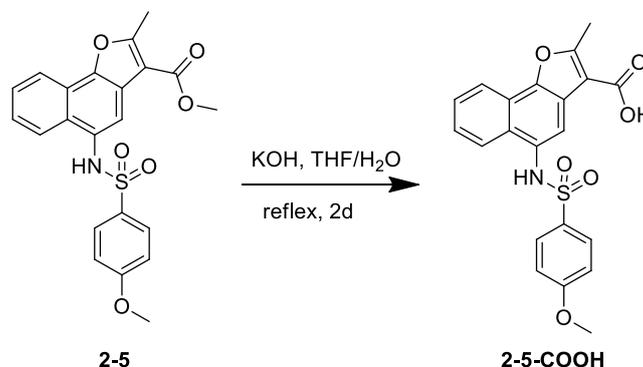
Compounds with more than one substituent or a fused ring system in the position of the phenyl ring of the benzenesulfonamide tended to have poor bioactivity (Table 4). The compounds available from SPECS and tested within the scope of this study only cover two or three methyl groups at different position of the benzene; other substituent groups like two or three methoxy groups were not measured. In addition, a compound including a tetracycline moiety (3-21) exhibited a low inhibitory activity on TNBC cells (Table S1).

**Table 4.** Inhibition rates of analogs of **2-5** and **2-8** identified by 2D similarity search (Part 2).

Compound	R <sup>4</sup>	R <sup>2</sup>	Inhibition%		
			MDA-MB-231	SUM-159	MCF-7
3-27		-OCH <sub>3</sub>	48.153	10.205	35.546
3-7		-OCH <sub>3</sub>	0.431	0.085	-4.733
3-6		-OCH <sub>2</sub> CH <sub>3</sub>	43.365	0.292	7.724
3-5		-OCH <sub>3</sub>	41.065	0.757	21.98
3-15		-OCH <sub>3</sub>	45.584	0.037	18.546
3-38		-OCH <sub>3</sub>	50.558	1.346	14.827
3-24		-OCH <sub>3</sub>	46.563	0.233	34.910
3-16		-OCH <sub>2</sub> CH <sub>3</sub>	48.171	0.732	43.439
3-35		-OCH <sub>2</sub> CH <sub>3</sub>	33.853	0.352	24.499
3-2		-OCH <sub>2</sub> CH <sub>3</sub>	24.255	-0.884	17.700
3-19		-OH	19.306	0.380	16.340
3-18		-OCH <sub>3</sub>	59.421	51.425	33.244

Ten compounds with good inhibition rates on both TNBC cell lines were selected for the measurement of IC<sub>50</sub> values on MDA-MB-231 and SUM-159 cell lines. However, the aqueous solubility of these compounds is poor. Hydrolysis of the ester in the R<sup>2</sup> position is expected to result in improved water solubility while maintaining biological activity. Therefore, we hydrolyzed **2-5** by refluxing it for two days under alkaline condition in the presence of an aqueous solution with potassium hydroxide and tetrahydrofuran (Scheme 1) to obtain **2-5-COOH**.

Most of the tested compounds inhibited both TNBC cell lines with similar strength (Table 5). Compound **2-5-COOH** had an approximately 10-fold reduced inhibitory activity compared to the ester compound (**2-5**). Its  $IC_{50}$  values were greater than 20  $\mu$ M for both cell lines.



**Scheme 1.** Hydrolysis route of Compound **2-5** to Compound **2-5-COOH**.

**Table 5.**  $IC_{50}$  for compounds measured on two TNBC cell lines.

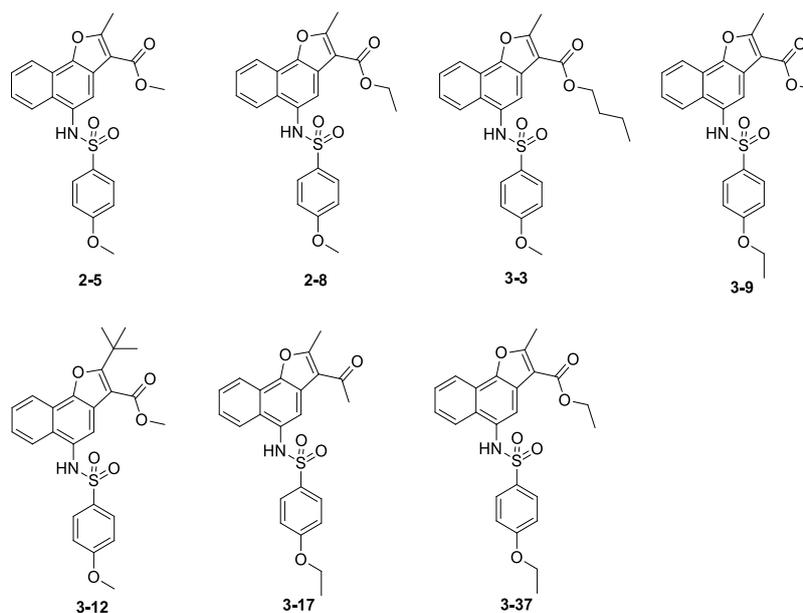
Compound	$IC_{50}$ ( $\mu$ M)	
	MDA-MB-231	SUM-159
2-5	3.12	2.91
2-8	2.96	2.50
3-3	3.25	8.66
3-9	2.68	2.66
3-12	11.91	11.53
3-17	1.77	1.94
3-18	5.20	24.15
3-20	10.04	16.97
3-28	>40	14.41
3-37	5.49	4.09
2-5-COOH	~24	24.25

Seven of the most potent compounds were chosen for evaluation of their bioactivity in further cell lines. In addition to three TNBC cell lines (MDA-MB-231, MDA-MB-436, and SUM-159) and the breast cancer cell line MCF-7, we employed the normal mammary epithelial cell line MCF-10A, which was used for testing the compounds for their effects on normal breast cells (Table 6). The results show that most of the compounds exhibited a weak inhibition of MCF-10A cells. Compound **3-17**, the most potent TNBC inhibitor identified in this study, also had the strongest inhibition on MCF-10A ( $IC_{50} = 0.66 \mu$ M). Thus, **3-17** may be less promising for the treatment of TNBC.

From a molecular structure point of view, the main differences of these compounds are the substituents of the phenyl ring of the benzenesulfonamide (**2-5**, **2-8**, **3-3**, and **3-12** are methoxy; **3-9**, **3-17**, and **3-37** are ethoxy), and/or naphtho[1,2-*b*]furan (Figure 5). Six compounds are esters, and Compound **3-17** is a ketone. Calculations suggest that **3-3** and **3-12** are less soluble in water, probably because of their longer carbon alkyl substituents (Table 6).

**Table 6.** IC<sub>50</sub> of compounds measured on different cell lines and calculated molecular properties.

Compound	IC <sub>50</sub> (μM)					Calculated Molecular Properties		
	MDA-MB-231	MDA-MB-436	SUM-159	MCF-7	MCF-10A	Molecular_Weight	AlogP	ADMET_Solubility_level
Tamoxifen	2.03	10.02	13.48	9.08	>40	371.5	6.319	1
2-5	3.12	1.99	2.91	>40	>40	425.5	3.514	2
2-8	2.96	2.80	2.50	>40	>40	439.5	3.863	2
3-3	3.25	3.36	8.66	ND <sup>a</sup>	>40	467.5	4.842	1
3-9	2.68	2.08	2.66	ND	>40	439.5	3.863	2
3-12	11.91	7.13	11.53	ND	>40	467.5	5.063	1
3-17	1.77	-0.01	1.94	ND	0.66	423.5	3.747	2
3-37	5.49	1.41	4.09	ND	>40	453.5	4.211	2

ND<sup>a</sup>: not determined.**Figure 5.** Structures of compounds in Table 6.

### 3. Materials and Methods

#### 3.1. Overall Protocol

3D screening methods taking into account the molecular shape and electrostatic maps of molecules were employed to identify compounds of interest in the SPECS compound library. Compounds were selected for purchase and experimental testing in cell-based assays taking into account their calculated aqueous solubility and structural diversity (assessed by a cluster analysis). An iterative 2D similarity search was conducted to follow up on active compounds and identify (further) derivatives for testing (Figure 6).

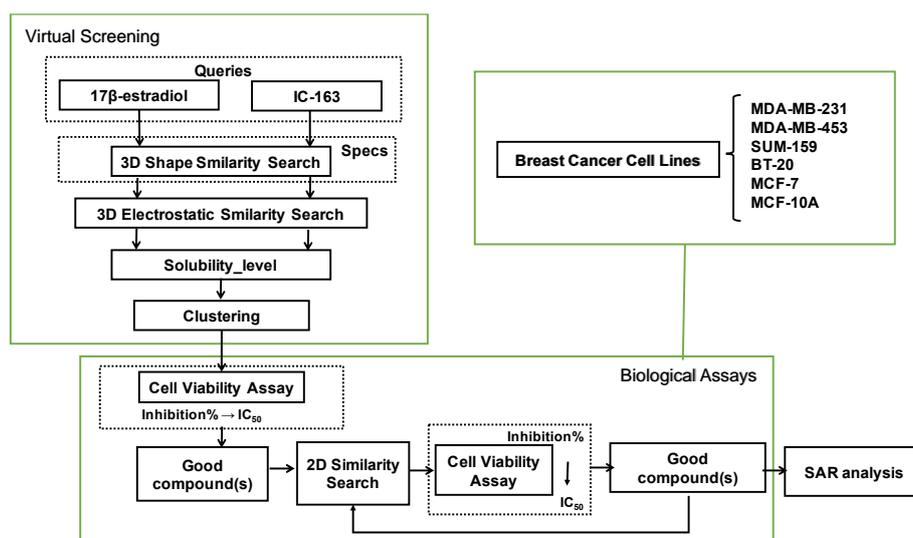


Figure 6. Workflow of virtual screening and bioassays.

### 3.2. Three-Dimensional Similarity Search

The SPECS compound library (<http://www.specs.net/>, accessed May 2014) was prepared with a workflow developed with Pipeline Pilot v7.5 (PP 7.5, Accelrys Software, Inc., San Diego, CA, USA.), in which minor salt components were removed and the chemical structures standardized. The prepared database was filtered with “Blockbuster” filter of FILTER (version 2.2.1, OpenEye Scientific Software, Inc., Santa Fe, NM, USA) to remove molecules with undesired physicochemical properties with respect to molecular weight, number of heavy atoms, and aqueous solubility. Next, the databases were processed with OMEGA [23] (version 2.4.5, OpenEye Scientific Software, Inc., Santa Fe, NM, USA) to generate up to 500 conformations for each molecule.

ROCS [17,24] (version 3.2.0, OpenEye Scientific Software Inc., Santa Fe, NM, USA) was employed for 3D shape comparison. The lowest energy conformers of 17β-estradiol and IC-163 generated with OMEGA served as input for screening with ROCS. EON was employed to re-rank the top-ranked molecules obtained with ROCS based on the similarity of electrostatic properties.

### 3.3. Water Solubility Prediction and Structure Cluster Analysis

Water solubility at 25 °C was calculated with the ADMET solubility prediction module of Discovery Studio 2.5 (Accelrys Software, Inc., San Diego, CA, USA). We removed molecules with ADMET solubility level in 0 (extremely low) and 1 (very low) to ensure that the chosen compounds have acceptable solubility. The remaining compounds were clustered based on ECFP\_6 and FCFP\_6 fingerprints to assist the selection of compounds for experimental testing.

### 3.4. Two-Dimensional Similarity Search

The most promising compounds, C10, 2-5, and 2-8, served as templates for 2D similarity search based on ECFP\_6 or FCFP\_6 fingerprints.

### 3.5. Cell Viability Assays

Four human TNBC cell lines (MDA-MB-231, MDA-MB-453, SUM-159, and BT-20), a non-TNBC breast cancer cell line (MCF-7) and a normal mammary epithelial cell line (MCF-10A) were cultured with DMEM (phenol free) supplemented with 2.5% CS-FBS and 1% L-Glu.  $1.2 \times 10^3$  of cells were seeded into 384-well microplates and maintained for 24 h in an incubator at 37 °C in a 5% CO<sub>2</sub>, saturated humidified atmosphere. Different compounds were added into cells with 9 concentrations

from 0.156 to 40  $\mu\text{M}$  for 72 h. Tamoxifen was the positive control. Then CCK-8 solution was added into cells and incubated for another 4 h. Absorbance was measured with a Microplate Reader at 450/600 nm. The  $\text{IC}_{50}$  values of the compounds on TNBC cell lines were derived using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA).

#### 4. Conclusions

3D and 2D similarity searches led to the identification of *N*-(naphtho[1,2-*b*]furan-5-yl)benzenesulfonamides as novel inhibitors of TNBCs. The most potent compounds (**2-5** and **2-8**) obtained  $\text{IC}_{50}$  values of 2–3  $\mu\text{M}$  on different TNBC cell lines and showed no inhibitory activity on normal (i.e. MCF-7 and MCF-10A) cells in vitro, indicating their selectivity against TNBC cells. These compounds and derivatives thereof could serve as starting points for further research and development of selective TNBC inhibitors.

**Supplementary Materials:** The following are available online. Figure S1: Structures of 32 compounds from 3D similarity search; Figure S2: Inhibition rates of 32 compounds at 10  $\mu\text{M}$  from 3D similarity search; Figure S3: Inhibition rates of 12 compounds at 5  $\mu\text{g}/\text{mL}$  from C10 2D similarity search; Figure S4: Inhibition rates of 40 compounds at 5  $\mu\text{g}/\text{mL}$  from Compounds **2-5** and **2-8** 2D similarity search; Table S1: Inhibition rates of analogs of **2-5** and **2-8** identified by 2D similarity search (Part 3).

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**Conflicts of Interest:** The authors declare that they have no conflict of interests.

#### Abbreviations

TNBC	triple-negative breast cancer
ER	estrogen receptor
PR	progesterone receptor
HER2	human epidermal growth factor receptor 2
VEGF	the vascular epidermal growth factor
3D	three-dimensional
ROCS	Rapid Overlay of Chemical Structures
2D	two-dimensional
ECFP <sub>6</sub>	extended connectivity fingerprints of maximum diameter 6
FCFP <sub>6</sub>	function class fingerprints of maximum diameter 6

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**Sample Availability:** Samples of the compounds 2-5, 2-8, 3-1 to 3-40 are available from the authors.



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