ORIGINAL ARTICLE

Nanomolar activity of 4-hydrazinylphenyl benzenesulfonate against breast cancer Michigan Cancer Foundation-7 cell lines

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ABSTRACT

Hydrazine is an alkaline reduction compound which is widely used in synthesis. Based on the structure–activity analysis, to elicit antitumor activity, the presence of the N-methyl group is an absolute requirement. The aim of the research is to synthesize a new hydrazine derivate compound that has potency as a novel anti-breast cancer. 4-hydrazinylphenyl benzenesulfonate was synthesized employing reduction and diazotization methods. Structure characterization was carried out using Fourier transform infrared (FTIR), C13-nuclear magnetic resonance (NMR), H1-NMR, and High Resolution Time-of-Flight Mass Spectrometry (HR-TOF-MS). The anti-cancer activity of this compound against breast cancer Michigan Cancer Foundation-7 (MCF-7) cell line was determined using a PrestoBlue viability assay. The new of hydrazine derivative, 4-hydrazinylphenyl benzenesulfonate, has been successfully synthesized. The reduction and diazotization methods have been successfully used in the synthesis of new compound of hydrazine derivatives. Structure characterization of 4-hydrazinylphenyl benzenesulfonate was established using FTIR, C13-NMR, H1-NMR, and HR-TOF-MS. The anti-cancer activity of this compound against breast cancer MCF-7 cell line was determined using a PrestoBlue viability assay with IC50 0.00246 μg/mL or 9.32 nM. In conclusion, 4-hydrazinylphenyl benzenesulfonate was successfully synthesized as a new candidate for anti-breast cancer compound.

Key words: Anti-breast cancer, hydrazine derivate, synthesis

INTRODUCTION

The leading cause of death in women is cancer. Breast cancer is the leading cause of women's death and the second leading cause of death in the world Siegel, Miller *et al*. 2019. [1] Early treatment of breast cancer most often uses tamoxifen, the antiestrogen for long-term treatment Chen, Chang *et al*. 2011.[2] The limitation therapy using tamoxifen, therapy that affects the endocrine system causes resistance after several months of use. However, 70%–80% give a positive response to tamoxifen therapy for breast cancer with ERα positif expression. Breast cancer with ERα positif occurs in about 70% of cases.[3] The emergence of resistance caused by tamoxifen therapy may be due to either a two-stage process of cell alteration or a simple selection of heterogeneous cells followed by cells affected by cytotoxic compounds.[4]

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Synthesis of heterocyclic compounds using hydrazine which has two amines is widely used for various purposes,[5] a precursor to polymerization, and pharmaceuticals.[6] Hydrazine and its derivatives show antidepressant properties in the biological application,[5] cause lung tumor, and prevent breast cancer adenocarcinomas in mice.[7] In another research study, hydrazine and hydrazide derivatives show higher antiproliferative activities or exhibited comparable than the control drug cisplatin.[8] However, hydrazine proved to act as a carcinogenic agent.[9] In the previous study, oral therapy using 60 mg hydrazine sulfate 1–4 times daily, in patients with a variety of solid tumors shown not a reduction of 50% tumor size, so in this study modification conduct with presence the N-methyl group.[10] Recently, several hydrazine derivatives have been found that have anti-breast cancer activity. There are several new compounds derived from phenylhydrazine which have anti-breast cancer effects (https://doi.org/10.4236/ijoc. 2022.121003). Substitution of hydrazine derivatives in novel celecoxib analog produces a potential anti-breast cancer agent (https://doi.org/10.2174/1573406418666220309123648). Based on the structure–activity analysis, to elicit antitumor activity, the presence of the N-methyl group is an absolute requirement. The toxicity of the compound can be reduced in the chemical stability occurs due to the electron withdrawing group para to the methylhydrazine moiety.[11] The aim of the research is to synthesize a new hydrazine derivate compound that has potency as a novel anti-breast cancer.

MATERIALS AND METHODS

Materials

All chemicals are used without prior purification (Merck, USA). Benzenesulfonyl chloride and 4-nitrophenol (Sigma-Aldrich, USA) were used as starter material. Reduction and diazotization reaction methods were used to synthesize the compound in the title. Na_2SO_3 (Sigma-Aldrich, USA) was used as a reductor in HCl concentrate solvent, and $NaNO_2$ in HCl was used in diazotization reaction.

Instrumentation

The instruments used in this research were Fourier transform infrared (FTIR) (IRPrestige-21, Shimadzu), HR-TOF-MS (Waters QTof MS Xevo), and nuclear magnetic resonance (NMR) (Agilent 500 MHz with system console DD2, CDCl3 as a solvent, and operate on frequency 500 MHz (^{1}H NMR) dan 125 MHz (^{13}C NMR)).

Methods

Synthesis of 4-hydrazinylphenyl benzenesulfonate

The first step was the synthesis of 4-nitrophenyl benzenesulfonate 2. 4-nitrophenol (5 mmol) and benzenesulfonyl chloride (5 mmol) mixed with 25-ml CH_3CN (Sigma-Aldrich, USA) as a solvent and NaOH (10 mmol) as a catalyst. The reaction was carried out in a microwave 300 watt. The reaction progress was monitored every 30 s using thin-layer chromatography (TLC) and was stopped when it was completed. The TLC spot was detected using ultraviolet light. The FTIR spectrum was used to know the characterization of 4-hydrazinylphenyl benzenesulfonate produced.

Nitro groups of 4-nitrophenyl benzenesulfonate 2 (5 mmol) were reduced using Na_2SO_3 (10 mmol) and 2.5 g HCl concentrate in an ice bath with stirring it for 1 h, thus amine (-NH_2) of 4-aminophenyl benzenesulfonate 3 was produced. The 4-aminophenyl benzenesulfonate 3 (5 mmol) further was reacted with $NaNO_2$ (10 mmol) and 25-mL HCl concentrate in 50 mL aquadest by stirring it for 2 h into an ice bath to produced 4-([phenylsulfonyl] oxy) benzenediazonium chloride 4. Na_2SO_3 as a reductor changed the 4-([phenylsulfonyl] oxy) benzenediazonium chloride 4-4-hydrazinylphenyl benzenesulfonate 5 in HCl concentrate by stirring it into an ice bath for an hour.

The cytotoxicity assay

Cell culture was prepared in Roswell Park Memorial Institute Medium containing fetal bovine serum 10%, 50 µL/50 mL ceftriaxone (200.000 ppm) (Invitrogen, USA). Cell culture in 96-well plates was incubated at 37°C and 5% CO_2 gas until 70% cell growth. A positive control was used by cisplatin and dimethyl sulfoxide (Shimadzu Aldrich, USA) as the negative control. Positive control, negative control, and sample were put in 96-well plates containing

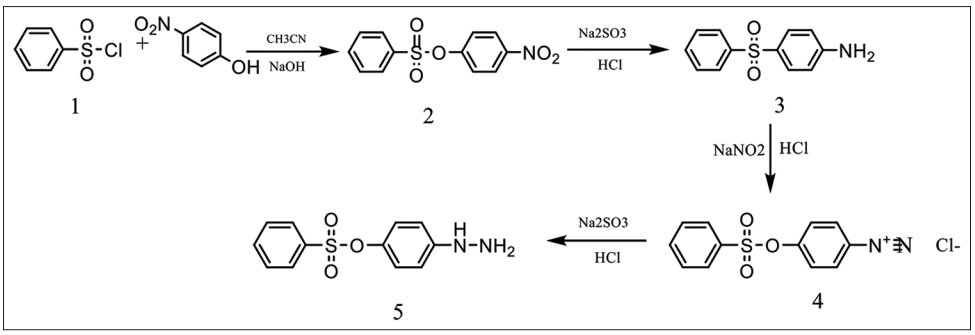

Figure 1: Synthesis scheme of the 4-hydrazinylphenyl benzenesulfonate 5

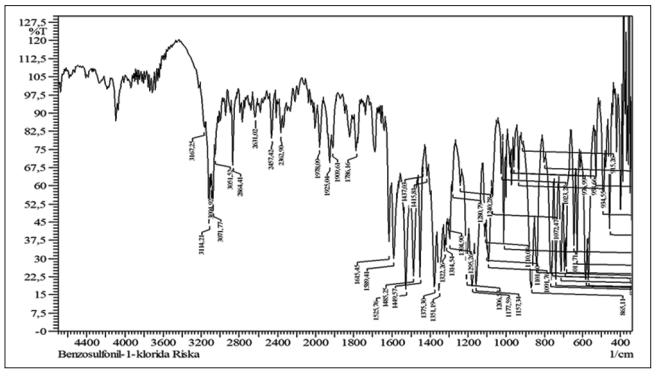

Figure 2: FTIR spectra for 4-nitrophenyl benzenesulfonate. FTIR: Fourier transform infrared

confluent cell culture and then incubation for 24 h at 37°C and 5% CO_2 gas. PrestoBlue cell viability reagent was put into each well in a microplate and further incubated for 1–2 h then there will be a color change, the absorbance will be measured. Absorbance was measured using multimode reader at 570 nm.

RESULTS

Synthesis of 4-hydrazinylphenyl benzenesulfonate 5

Figure 1 shows the synthesis scheme of the 4-hydrazinylphenyl benzenesulfonate 5.

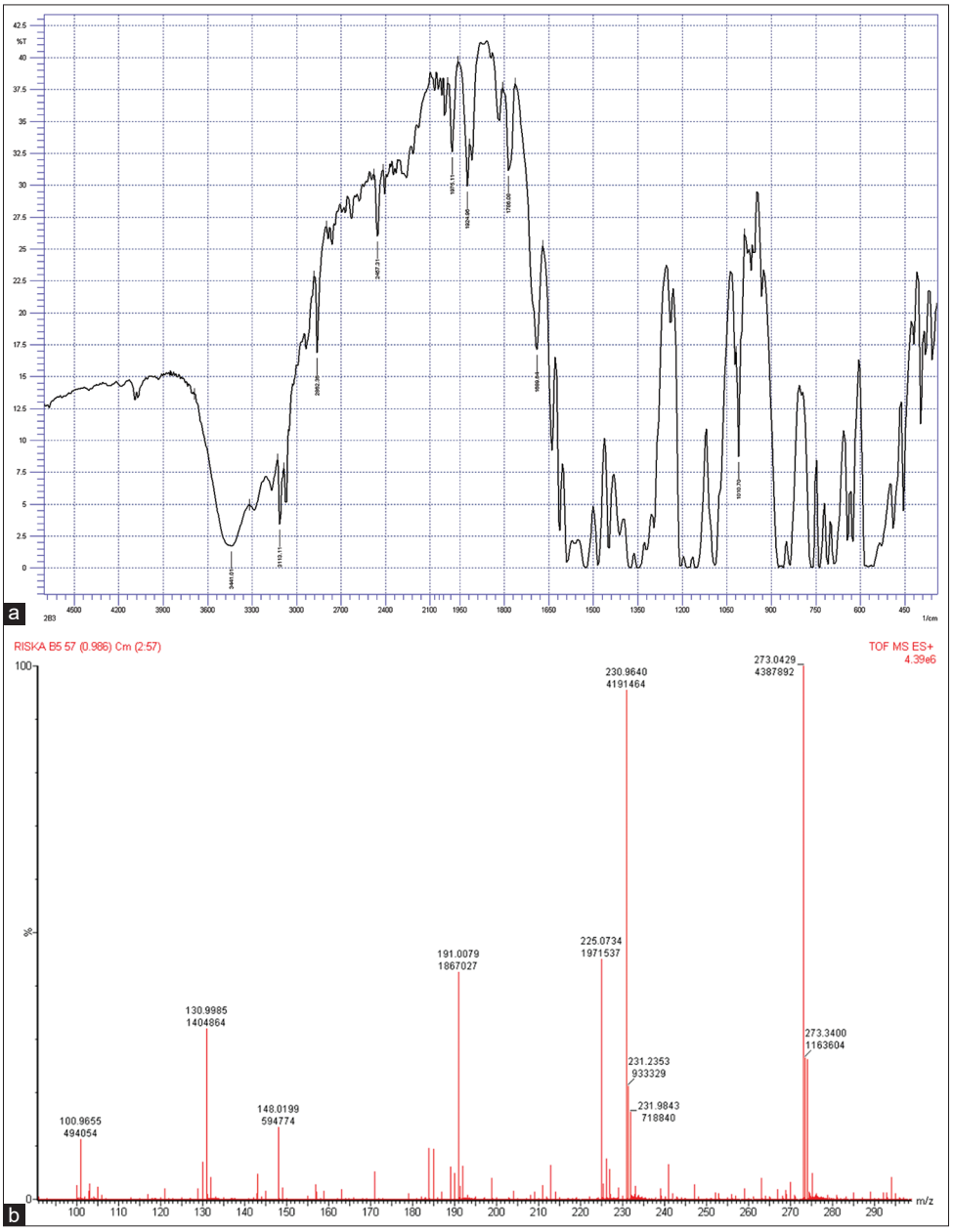

Figure 3: FTIR spectra (a) and mass spectra (b) for 4-aminophenyl benzenesulfonate. FTIR: Fourier transform infrared

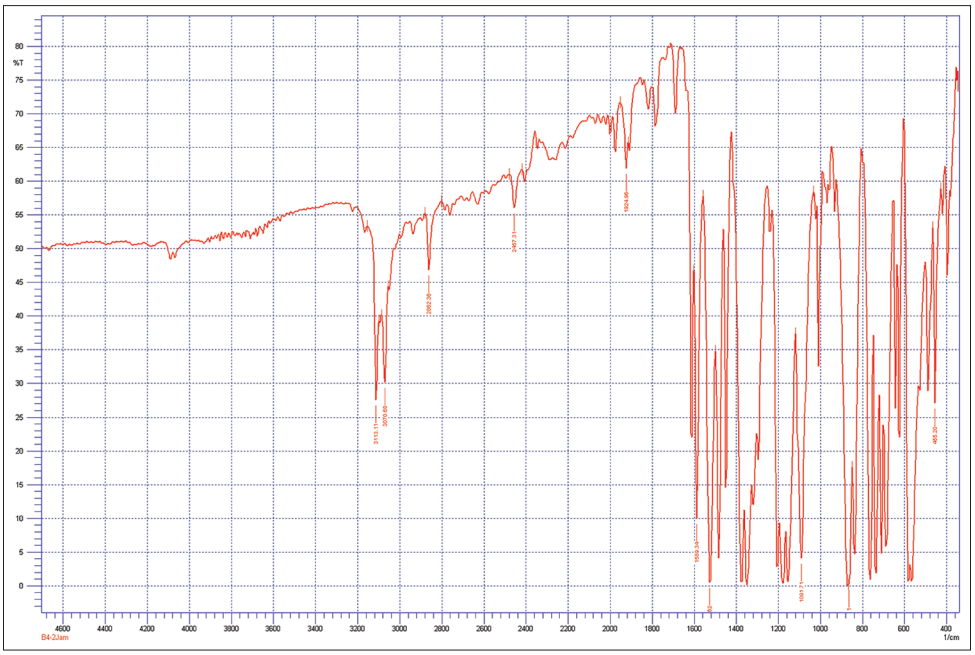

Figure 4: The FTIR spectra of 4-([phenylsulfonyl] oxy) benzene diazonium chloride 4. FTIR: Fourier transform infrared

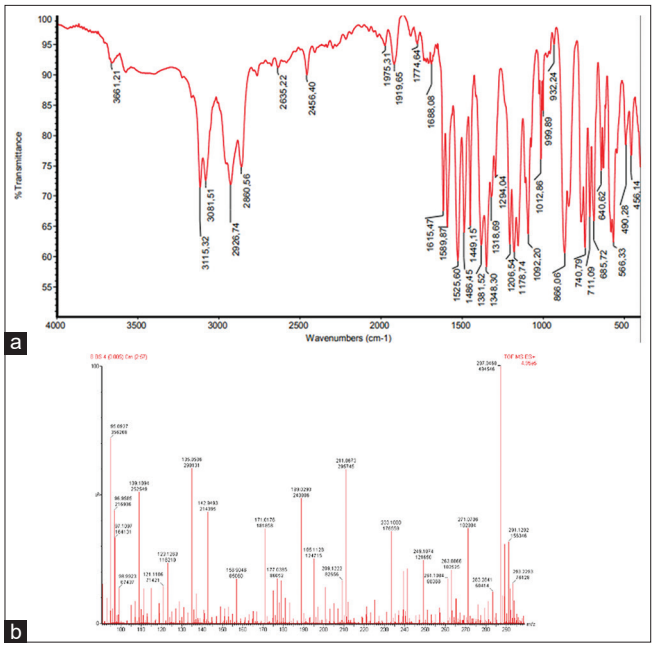

Figure 5: FTIR spectra (a) and mass spectra (b) of 4-hydrazinylphenyl benzenesulfonate. FTIR: Fourier transform infrared

The product of synthesis of the first step in Figure 1 has been characterized by the FTIR spectrum. Figure 2 shows FTIR spectra for 4-nitrophenyl benzenesulfonate.

The FTIR absorption of 4-aminophenyl benzenesulfonate 3 is shown in Figure 3a, whereas the mass spectrum (HR-TOF-MS) for 4-aminophenyl benzenesulfonate 3 is shown in Figure 3b.

The FTIR spectrum of 4-([phenylsulfonyl] oxy) benzenediazonium chloride 4 is shown in Figure 4.

Table 1: NMR data of the 4-hydrazinylphenyl benzenesulfonate in $CDCl_3$

Number of atom [Figure 2]	δ_H (ppm) (mult, J Hz)	δ_C (ppm)	HMBC
1, 1′	7.18 (d, J=9.1 Hz, 2H)	123.32	C-1, C-1′, C-7, C-8
2, 2′	8.18 (d, J=2.05, 6.85 Hz, 2H)	125.55	C-2, C-2′, C-7, C-8
3, 3′	7.86 (d, J=2.6, 7.1 Hz, 2H)	128.56	C-3, C-3′, C-5
4, 4′	7.58 (t, J=0.2, 8.2 Hz, 2H)	129.62	C-3, C-3″, C-4, C-4′, C-6
5		134.92	
6	7.72 (t, J=2.6, 7.5 Hz, 1H)	135.00	C-3, C-3′
7		146.39	
8		153.91	

HMBC: Heteronuclear Multiple Bond Correlation

Figure 5a shows the FTIR spectra of 4-hydrazinylphenyl benzenesulfonate, and the mass spectrum which is suitable for 4-hydrazinylphenyl benzenesulfonate 5 is shown in Figure 5b. The numbering structure for the 4-hydrazinylphenyl benzenesulfonate 5 is shown in Figure 6.

The NMR spectrum of the title compound for 4-hydrazinylphenyl benzenesulfonate (5) which has eight different chemical environments is shown in Figures 7-9. Table 1 shows the NMR data of the 4-hydrazinylphenyl benzenesulfonate 5 in $CDCl_3$.

Viability assay

Figure 10 shows the curve of growth inhibitory (%)

versus concentration of 4-hydrazinylphenyl benzenesulfonate (5) (μg/mL) treatment in MCF-7.

DISCUSSION

Synthesis of 4-hydrazinylphenyl benzenesulfonate 5

The synthesis of 4-hydrazinylphenyl benzenesulfonate 5 using reduction and diazotization method[12,13] has been done following the reaction process in Figure 1. First step in this synthesis is produce 4-nitrophenyl benzenesulfonate which has been characterized by the FTIR spectrum. Ether groups were detected at 1300–1000/cm, and strong absorption at 1600–1530/cm and 1390–1300/cm was indicated for the nitro group. That was suitable for the 4-nitrophenyl benzenesulfonate 2 compound [Figure 2].

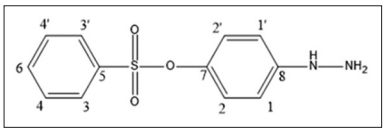

Figure 6: The numbering structure of 4-hydrazinylphenyl benzenesulfonate 5

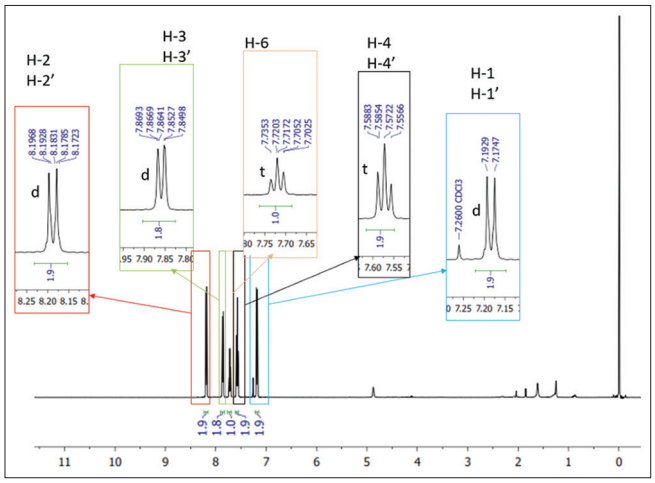

Figure 7: [1]H-NMR for 4-hydrazinylphenyl benzenesulfonate. NMR: Nuclear magnetic resonance

The 4-aminophenyl benzenesulfonate 3 produced from 4-nitrophenyl benzenesulfonate 2 reduction. The FTIR spectrum of 4-aminophenyl benzenesulfonate 3 showed strong absorption in wave number 3440/cm for the NH_2 [Figure 3a], whereas the mass spectrum (HR-TOF-MS) was m/z = 273.0429 (M + H + Na) which is suitable for 4-aminophenyl benzenesulfonate 3 [Figure 3b].

The FTIR spectrum of 4-([phenylsulfonyl] oxy) benzenediazonium chloride 4 showed the peak at wave number 3100/cm for CH-benzene of the diazonium salt 4 which produced from hydrazine reaction of 4-aminophenyl benzenesulfonate 3 [Figure 4].

Double peak at wave number 3661.21/cm [Figure 5a] for-NH- and the mass spectrum was m/z = 287.0460 (M+Na) [Figure 5b] which is suitable for 4-hydrazinylphenyl benzenesulfonate 5.

The NMR spectrum of the title compound 4 hydrazinylphenyl benzenesulfonate 5 shows the 1 H NMR and 13C NMR spectrums as Figures 7-9, there are suitable for 4 hydrazinylphenyl benzenesulfonate (5) which has eight difference chemical environments.

Based on the NMR spectrum, it was confirmed that 4-hydrazinylphenyl benzenesulfonate 5 was successfully synthesized.

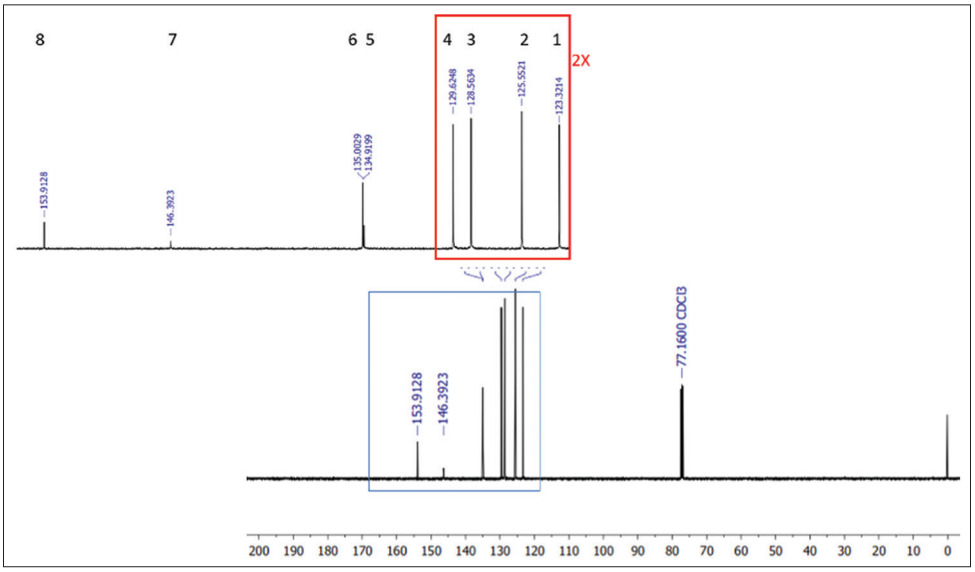

Figure 8: [13]C-NMR for 4-hydrazinylphenyl benzenesulfonate. NMR: Nuclear magnetic resonance

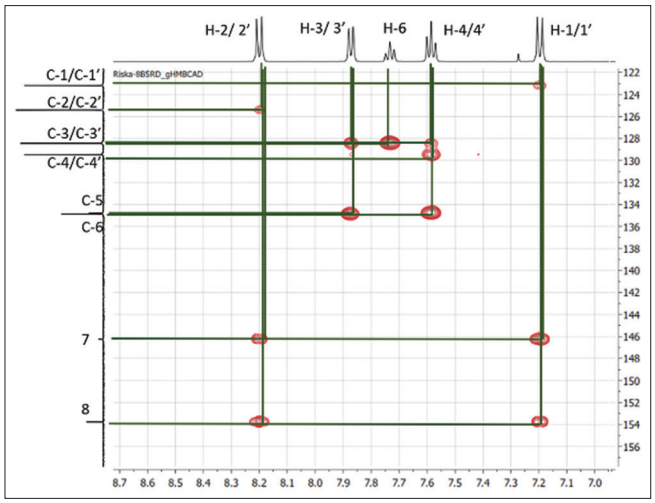

Figure 9: The HMBC spectra of 4-hydrazinylphenyl benzenesulfonate. HMBC: Heteronuclear Multiple Bond Correlation

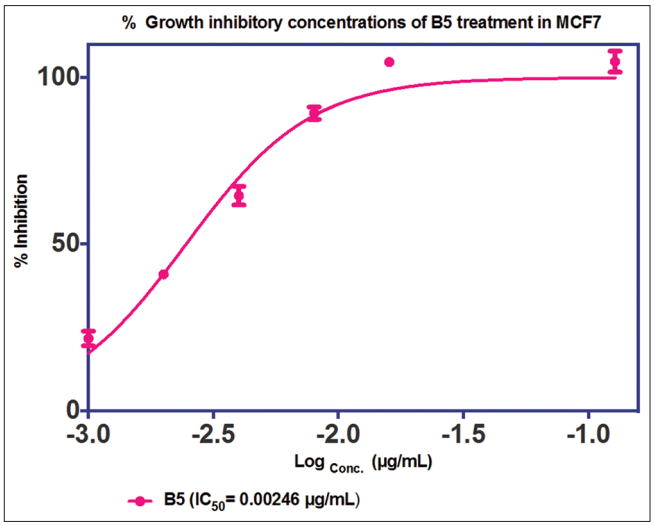

Figure 10: Curve of growth inhibitory (%) versus concentration of 4-hydrazinylphenyl benzenesulfonate (5) (µg/mL) treatment in MCF-7. MCF-7: Michigan Cancer Foundation-7

Viability assay

The development of hydrazine derivative drugs has been widely carried out as guide compound in medicine.[14] Pharmaceutical companies use cell-based assays for better test results and screening of cytotoxic compounds, recently. The increasing use of cell-based assays contributes to improving the simple method that correlates with *in vivo* data.[15] Cell proliferation was used to determine the effect of toxic compounds on cells, while cell viability was used to determine the number of healthy cells. In general, the same method is used to determine cell viability and proliferation. Screening to determine of the cytotoxicity of the test compounds generally using cell cytotoxicity and proliferation assay.[16] The viability or antiproliferative assay was conducted using the PrestoBlue cell viability reagent for breast cancer Michigan Cancer Foundation-7 cell line (ATCC® HTB-22™). PrestoBlue is a reliable test method to determine cytotoxicity and cell viability.[17,18] Resazurin based viability assay is the new more rapid and effficient approach that has clear advantages, shows lower variability of dose–response curves.[18] Cytotoxicity of the 4-hydrazinylphenyl benzenesulfonate 5 was a strong level with IC_{50} = 0.00246 µg/mL or 9.32 nM as shown in Figure 10.

CONCLUSION

4-hydrazinylphenyl benzenesulfonate (5) was successfully synthesized as a new candidate for anti-breast cancer compound with IC_{50} 0.00246 µg/mL.

Acknowledgments

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Author contributions

Riska Prasetiawati, Adel Zamri, and Muchtaridi Muchtaridi performed the experiments. Muchtaridi Muchtaridi and Adel Zamri, conceived and designed the experiments. Riska Prasetiawati, Adel Zamri, and Muchtaridi M analyzed the data. Riska Prasetiawati and Muchtaridi Muchtaridi wrote the article. Muchtaridi M collected the funding.

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Conflicts of interest

There are no conflicts of interest.

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