

## Original Research Article

# Synthesis and cytotoxicity studies on new pyrazole-containing oxime ester derivatives

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### Abstract

**Purpose:** To synthesize a series of new 1-(2-naphthyl)-2-(1H-pyrazol-1-yl)ethanone oxime ester derivatives (**5-12**) with potential anticancer properties, and to determine their cytotoxic effects in mouse fibroblast and human neuroblastoma cell lines.

**Methods:** The title compounds were obtained through sodium salt reaction of 1-(naphthalene-2-yl)-2-(1H-pyrazol-1-yl)ethanone oxime (**4**) with various acyl chlorides. The cytotoxic effects were evaluated by MTS colorimetric assay, while physicochemical descriptors were calculated using QikProp software.

**Results:** Most of the compounds showed approximately 50 – 60 % inhibition against SH-SY5Y neuroblastoma cells at 100 µM. Of these, compound **7a** was the most active combination with an IC<sub>50</sub> value of 85.94 µM. The toxic effect of the compounds on mouse fibroblast cell line was insignificant ( $p < 0.05$ ) even when the dose was increased. The calculated physicochemical properties of the compounds were within drug-like chemical space.

**Conclusion:** The synthesized oxime ester derivatives with pyrazole ring exhibit selective toxicity to neuroblastoma cells without affecting healthy mouse fibroblast cells. The compounds proved to be drug-like while their pharmacokinetic features were also encouraging, and were in line with *in silico* predictions.

**Keywords:** Cytotoxic activity, E/Z isomer, Neuroblastoma cell, Oxime ester, Pyrazole

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## INTRODUCTION

Neuroblastoma is an aggressive malignant tumor that originates in the early nerve cells of the sympathetic nervous system. Neuroblastoma often occurs in childhood and gives limited response to chemotherapy. The tumor is usually found to have metastasized by the time a

diagnosis is made; as a result, survival rate is low in children with advanced neuroblastoma. Besides, the metastasized tumor commonly leads to unwanted side effects during chemotherapy. For these reasons, new and effective anticancer drugs need to be developed against neuroblastoma [1,2].

Heterocyclic compounds have been in use in medicinal chemistry as basic structures in the development of active molecules, many of which contain pyrazoles or pyrazole substitutes [3-6]. In addition, several effective molecules also contain oxime and oxime ester groups [7,8]. For example, some 2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-1-arylethanone derivatives are reported to be effective anticancer agents [8,9]. One of our own earlier studies, too, have shown cytotoxic activity in certain 3,5-dimethylpyrazole derivatives [10]. Building on the existing research results, this study analyses the 1-arylethanonepyrazole structure as a scaffold along with 12 new compounds containing oxime ester group. In order to evaluate their efficacy and selectivity against neuroblastoma cells, the preparations are tested *in vitro* for their cytotoxic effects against SH-SY5Y (human neuroblastoma) and L929 (mouse fibroblast) cell lines.

Physicochemical and pharmacokinetic profiles play a key role in the success of investigational compounds in the market, especially in studies on anticancer drug design [11]. To this end, our study also calculates the number of physicochemical and pharmacokinetic descriptors related to ADMET (Absorption, Distribution, Metabolism, Elimination, and Toxicity) properties.

## EXPERIMENTAL

### Chemistry

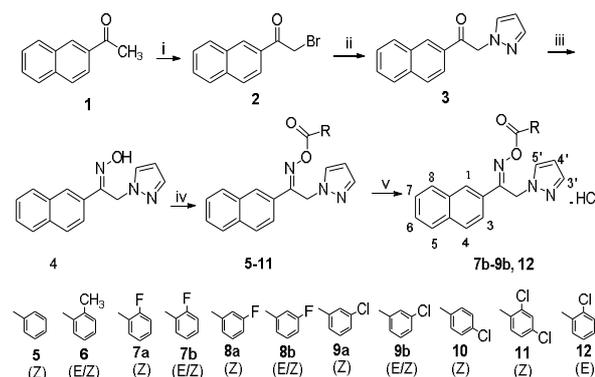
All reagents and solvents in this study were obtained from Aldrich, Merck, and Carlo Erba. All reactions were observed with analytical thin-layer chromatography using Merck Kieselgel 60 F<sub>254</sub> plates. NMR spectra of the compounds were recorded with Bruker Avance 300 MHz Ultrashield™ NMR Spectrometers. All chemical shifts ( $\delta$ ) are reported in parts per million (ppm) using TMS as the internal reference. IR spectra were recorded with a Perkin Elmer Spectrum One FT-IR spectrophotometer using the attenuated total reflectance (ATR) FT-IR method. Elemental analyses were performed on a Leco CHNS-932 elemental analyzer.

2-Bromo-1-(naphthalene-2-yl)ethanone (**2**), 1-(naphthalene-2-yl)-2-(1*H*-pyrazol-1-yl)ethanone (**3**), 1-(naphthalene-2-yl)-2-(1*H*-pyrazol-1-yl)ethanone oxime (**4**) were all synthesized in line with the methods existing in the literature [12-14].

### General synthesis of the compounds (5-12)

0.011 mol metallic sodium (Na<sup>o</sup>) was dissolved in 10 ml absolute ethanol and 0.01 mol **4** was

added; the mixture was heated under reflux for 1h and EtOH was evaporated. Then, 0.01 mol appropriate acyl chloride was added to the residue including sodium oximate. The mixture was dissolved in dry dichloromethane (DCM) and stirred for 12 h at room temperature. DCM was then evaporated and the acquired residue was treated with ethyl acetate. In the next step, the precipitate was filtered off, the solute was evaporated to dryness, and the residue was washed with diethyl ether. At this stage, the compounds were converted to HCl salts using ethereal solution of gaseous HCl (gHCl) (Figure 1).



**Figure 1:** Synthetic routes and molecular structures of compounds **5-12**. Reagents and conditions are as follows: (i) CH<sub>3</sub>COOH, HBr, Br<sub>2</sub>, 0-5 ° C to rt (ii) pyrazole, Dimethylformamide, 0-5 ° C to rt (iii) NH<sub>2</sub>OH.HCl, EtOH, Ph ≈ 14, reflux (iv) EtOH, Na<sup>o</sup>, RCOCl, DCM, rt (v) gHCl

### Cytotoxic activity

The initial aim of our study is to determine the cytotoxic effects of the compounds on human neuroblastoma cell line (SH-SY5Y) and mouse fibroblast cell line (L-929) by performing the MTS assay protocol (CellTiter 96 Single Solution, Promega, Madison, Wis.). To this end, the cells were stored in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % (v/v) heat-inactivated Fetal Bovine Serum (FBS) by Gibco. The cells were seeded 24h before the treatment with compounds of the attached cells into 96-well plate at a density of 5 x 10<sup>3</sup> cells per well.

The compounds were then solubilized to 0.1 % as the final concentration form in dimethyl sulfoxide (DMSO). The medium at hand was replaced with fresh medium-containing compounds in 10, 25, 50 and 100 μM 10 % concentrations after 24 h of growth. Throughout the study vincristine was used as the positive control and the acquired medium as the negative control. Triplicate wells were prepared for the administration of each compound concentration.

The cells with the compounds were incubated at 5 % CO<sub>2</sub> atmosphere at 37 °C for 48 h. At length, the MTS solution was added and the incubation of the cells was continued at 37 °C for 1 - 3 h. The absorbance of the cells was measured in an ELISA reader at 490 nm. The percentage of cell viability in test results was considered as the ratio of absorbance of the treated cells to the untreated control cells. In order to observe average cell viability, the results of the three experiments were averaged. To this extent, the concentration (µM) of the compounds causing 50% cell death relative to the control cultures yields the IC<sub>50</sub> value. The IC<sub>50</sub> values were calculated in accordance with the inhibition rates using GraphPad Prism 7.02 [15].

### Molecular modelling

The ligands were sketched using 2D-Sketcher of Maestro (2018-1, Schrödinger, LLC, New York, NY, 2018), modelled and optimized using MacroModel (2018-1, Schrödinger, LLC, New

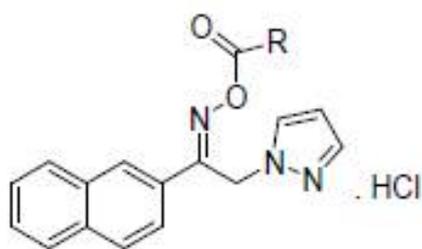
York, NY, 2018). OPLS\_2005 force field [16] QikProp was run in normal mode to calculate the descriptors throughout.

## RESULTS

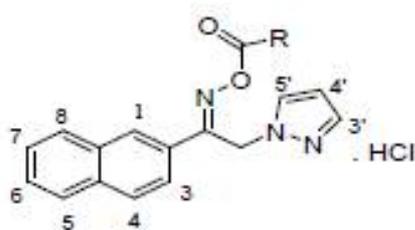
### Synthesis and spectral data

1-Naphthalene-2-yl-2-bromoethanone (**2**) was prepared by brominating 2-acetylnaphthalene (**1**). Compound **2** was converted to its ketone derivative (**3**) by *N*-alkylation of pyrazole. Then, compound **3** was converted to its oxime derivate (**4**) by refluxing with hydroxylamine hydrochloride (NH<sub>2</sub>OH.HCl) under alkaline conditions. The final compounds (**5-12**) were synthesized by sodium salt reaction of 1-(naphthalene-2-yl)-2-(1*H*-pyrazol-1-yl)ethanone oxime (**4**) with various acyl chlorides (**Figure 1**). The structures of **5-12** were determined by IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, and further elemental analyses (Tables 1 - 3).

**Table 1:** Chemical and physical data of compounds **5-12**



Compound	Config.	R	Yield (%)	Mp (°C)	Molecular formula	Analyte (%) calculated/found		
						C	H	N
5	Z	phenyl	35.35	153-4	C <sub>22</sub> H <sub>17</sub> N <sub>3</sub> O <sub>2</sub> .1/7CH <sub>2</sub> Cl <sub>2</sub>	72.36	4.74	11.43
6	<i>E/Z</i>	2-methylphenyl	47.61	150	C <sub>23</sub> H <sub>19</sub> F <sub>3</sub> N <sub>3</sub> O <sub>2</sub>	72.92	4.57	11.80
						74.78	5.18	11.37
7a	Z	2-fluorophenyl	21.39	139-41	C <sub>22</sub> H <sub>16</sub> FN <sub>3</sub> O <sub>2</sub> .1/8CH <sub>2</sub> Cl <sub>2</sub>	69.20	4.27	10.94
						69.14	4.18	11.22
7b	<i>E/Z</i>	2-fluorophenyl	10.10	132	C <sub>22</sub> H <sub>17</sub> ClFN <sub>3</sub> O <sub>2</sub>	64.47	4.18	10.25
						64.59	4.28	10.27
8a	Z	3-fluorophenyl	26.24	128-130	C <sub>22</sub> H <sub>16</sub> FN <sub>3</sub> O <sub>2</sub> .1/12CH <sub>2</sub> Cl <sub>2</sub>	69.72	4.28	11.04
						69.80	4.08	11.32
8b	<i>E/Z</i>	3-fluorophenyl	7.67	120-1	C <sub>22</sub> H <sub>17</sub> ClFN <sub>3</sub> O <sub>2</sub>	64.47	4.18	10.25
						64.71	4.24	10.48
9a	Z	3-chlorophenyl	23.87	130-2	C <sub>22</sub> H <sub>16</sub> ClN <sub>3</sub> O <sub>2</sub>	67.78	4.14	10.78
						67.53	4.14	10.59
9b	<i>E/Z</i>	3-chlorophenyl	6.48	108	C <sub>22</sub> H <sub>17</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>2</sub>	61.99	4.02	9.86
						62.84	4.13	9.63
10	Z	4-chlorophenyl	74.08	172-4	C <sub>22</sub> H <sub>16</sub> ClN <sub>3</sub> O <sub>2</sub> .2CH <sub>2</sub> Cl <sub>2</sub>	51.50	3.60	7.51
						51.32	3.36	7.64
11	Z	2,4-dichlorophenyl	20.51	126-7	C <sub>22</sub> H <sub>15</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>2</sub> .1/6CH <sub>2</sub> Cl <sub>2</sub>	60.73	3.53	9.58
						61.00	3.59	9.67
12	<i>E</i>	2-chlorophenyl	40.97	144-5	C <sub>22</sub> H <sub>17</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>2</sub> .1CH <sub>2</sub> Cl <sub>2</sub>	54.04	3.75	8.22
						54.32	3.71	8.45

**Table 2:**  $^1\text{H-NMR}$  data of compound **5-12** ( $\text{DMSO-}d_6$ , 300 MHz)

Comp.	-CH <sub>3</sub>	-CH <sub>2</sub> -N (2H; s)		H <sup>4</sup> (1H; t)	H <sup>3</sup> (1H; d)	Naphthalene aromatic H	H <sup>5</sup> (1H; s)
		E	Z				
5		-	5.94	6.18	7.39	7.57-8.18	8.43
6	2.32 (3H; s)	5.62 (78.13%)	5.87 (21.87%)	6.15	7.37	7.14-8.01	8.06
7a		-	5.89	6.17	7.37	7.41-8.13	8.39
7b		5.63 (47.40%)	5.89 (52.60%)	6.16	7.25	7.28-8.09	8.40
8a		-	5.96	6.18	7.39	7.57-8.03	8.44
8b		5.65 (48.78%)	5.96 (51.22%)	6.18	7.39	7.43-8.15	8.43
9a		-	5.96	6.19	7.40	7.57-8.15	8.45
9b		5.66 (67.11%)	5.96 (32.89%)	6.19	7.39	7.47-8.17	8.45
10		-	5.95	6.17	7.38	7.58-8.18	8.43
11		-	5.87	6.17	7.37	7.38-8.07	8.41
12		5.62	-	6.11	7.37	7.33-7.91	8.06

**Table 3:** IR and  $^{13}\text{C-NMR}$  Spectral data of the compound **5-12**

Compd	IR (ATR) C=O (cm <sup>-1</sup> )	$^{13}\text{C-NMR}$ ( $\text{DMSO-}d_6$ , 300 MHz)	
5	1742	46.76, 105.62, 124.00, 126.82, 127.59, 128.67, 129.03, 129.64, 131.12, 132.30, 133.72, 134.01, 139.47, 161.86, 162.69	
6	1740	20.67, 54.16, 105.62, 123.98, 124.90, 125.99, 126.74, 127.48, 127.54, 128.08, 129.63, 131.24, 131.66, 131.79, 131.99, 132.53, 133.00, 139.32, 139.45, 139.66, 163.03, 163.76	
7a	1733	46.83, 105.68, 116.44, 117.12, 123.98, 126.84, 127.59, 128.12, 128.66, 129.29, 131.11, 132.07, 133.73, 135.94, 139.50, 160.40, 162.20, 162.79	
7b	1733	46.82, 54.07, 117.12, 123.98, 124.50, 126.72, 127.72, 128.39, 128.66, 131.12, 131.47, 132.25, 133.11, 133.73, 135.78, 139.50, 162.20, 162.79, 163.25	
8a	1742	46.84, 105.64, 116.05, 120.92, 123.97, 125.89, 126.84, 128.15, 128.50, 129.50, 130.20, 131.10, 132.29, 133.75, 139.46, 161.72, 162.28, 163.66	
8b	1751	46.84, 53.96, 123.97, 125.10, 125.26, 125.89, 126.83, 127.14, 127.59, 128.02, 128.15, 128.39, 128.51, 128.69, 129.49, 131.12, 131.96, 132.29, 133.15, 133.75	
9a	1742	46.88, 105.65, 123.95, 126.85, 127.59, 128.16, 128.53, 129.07, 129.50, 130.05, 131.01, 132.30, 133.76, 139.45, 161.62, 162.31	
9b	1756	53.91, 105.68, 123.95, 125.07, 126.01, 127.08, 127.54, 127.88, 128.14, 128.31, 128.42, 128.70, 129.06, 129.97, 130.63, 130.96, 131.10, 131.95, 133.12, 133.60, 133.75	
10	1739	46.76, 105.62, 123.97, 126.85, 127.58, 128.13, 128.48, 128.68, 129.20, 129.50, 131.18, 131.48, 132.28, 133.73, 138.97, 139.48, 161.95, 162.15	
11	1761	46.89, 105.64, 123.92, 127.06, 127.59, 127.88, 128.16, 128.52, 129.22, 130.61, 131.21, 132.25, 132.94, 133.60, 133.76, 137.93, 139.52, 161.11, 162.44	
12	1754	54.06, 105.68, 124.85, 126.75, 127.40, 127.58, 128.32, 130.69, 131.24, 131.68, 131.87, 133.06, 135.51, 139.69, 162.26, 163.68	

For the purification of compounds **5-12**, the residue obtained by evaporation of DCM was first extracted from ethyl acetate, in which sodium chloride precipitated and the oxime ester derivatives dissolved. The ethyl acetate was then evaporated and the resulting residue was treated with diethyl ether, on which most of the derivatives solidified. The majority of the precipitated materials following diethyl ether

washing were in *Z* configuration. The ethereal solution was treated with gHCl to convert the dissolved derivatives to their salt forms. The application gave compound **12** as pure *E* and compounds **7b**, **8b**, **9b** as *E/Z* mixtures. This suggests that gHCl could trigger interconversion of diastereomers depending on several factors such as solvation, temperature etc.

The *E/Z* configuration of each compound was determined according to the chemical shift of singlet of methylene protons (-CH<sub>2</sub>-N) between the oxime ester group and the pyrazole ring. The -CH<sub>2</sub>-N protons of compound 6, for instance, were observed as two singlets with a down field peak at 5.87 ppm and an up field peak at 5.62 ppm. At this point, the differences in the chemical shift values were dependent on the angle between the C-H bond and the C=N-OH plane. The proton, sharing the same side with the aromatic group, shifted to higher ppm because of the anisotropic effect of the aromatic group. Here, the intensities of the two singlet peaks were 21.87% and 78.13%, respectively. The intensity values were calculated by the ratio of peak integrals. These configurations of the compounds (*E* or *Z*) as well as their chemical shifts were also calculated (Table 4).

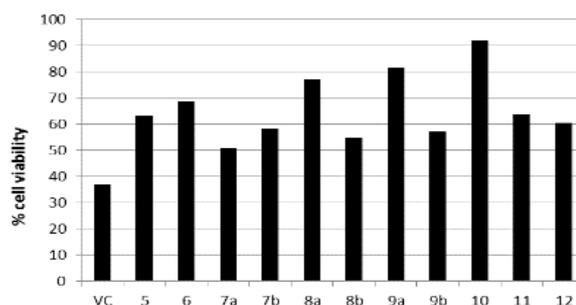
Since the synthesized compounds were  $\beta$ -naphthyl derivatives, the protons (H<sup>1</sup> and H<sup>2-8</sup>) of the naphthalene ring were observed between 7.0 - 8.2 ppm in accordance with the results of existing research in the literature. The H<sup>4</sup> proton of the pyrazole was observed in triplets at about 6.11-6.19 ppm, while H<sup>3</sup> protons were observed in the aromatic field. Yet, the pyrazole H<sup>5</sup> was seen between 8.06-8.45 ppm. This shift was due to the configuration of the structure as it was affected by the electronegativity of the oxygen.

### *In vitro* cytotoxic activity

Cytotoxic and anticancer effects of the synthesized compounds were tested at four concentrations (10, 25, 50 and 100  $\mu$ M) and the IC<sub>50</sub> values were determined accordingly. The results of cytotoxic activity against SH-SY5Y (human neuroblastoma) and L929 (mouse fibroblast cell line) cells are shown in Table 5.

According to this, the compounds were effective against the SH-SY5Y cell line. In addition, they

did not show significant toxicity against healthy fibroblast cells despite increasing doses, which indicates selectivity towards cancerous cells (Table 5). 2-fluorobenzoyl derivative (**7a**) was the most active compound throughout with a potency comparable to the positive control, vincristine. This compound inhibited approximately 50% of cancerous cells at 100  $\mu$ M (IC<sub>50</sub> = 85.94  $\mu$ M) (Figure 2). **7a** also presented the best selectivity index against the SH-SY5Y cell line with a SI value of 9.33 as shown in Table 5.



**Figure 2:** Cell viability plot of SH-SY5Y cell line treated with **5-12** and Vincristine (VC) at 100 $\mu$ M

### Evaluation of drug-like chemical space

QikProp (2018-1, Schrödinger, LLC, NY, 2018) calculates 40 descriptors determined for physicochemical and pharmacokinetic profiling. QikProp also provides an ideal value range for each descriptor based on an experimental database of 95 % of a compound set of 500 known drugs [17,18].

In our study, QikProp was run only for pure isomers (**5**, **7a**, **8a**, **9a**, **10-12**) and a part of these results are presented in **Table 6**. For a complete list of results and descriptions of all descriptors, please see the additional information.

**Table 4:** Intensities and chemical shifts of -CH<sub>2</sub>-N protons of each diastereomer

Compound	E		Z	
	Chemical shift (ppm)	Intensity (%)	Chemical shift (ppm)	Intensity (%)
<b>5</b>	-	-	5.94	100
<b>6</b>	5.62	78.13	5.87	21.87
<b>7a</b>	-	-	5.89	100
<b>7b</b>	5.63	47.40	5.89	52.60
<b>8a</b>	-	-	5.96	100
<b>8b</b>	5.69	48.47	5.96	51.22
<b>9a</b>	-	-	5.96	100
<b>9b</b>	5.66	67.11	5.96	32.89
<b>10</b>	-	-	5.95	100
<b>11</b>	-	-	5.87	100
<b>12</b>	5.62	100	-	-

**Table 5:** Cell viability (%) of SH-SY5Y and L929 cell line treated with **5-12** and vincristine

Compound	Cell line	10 $\mu$ M	25 $\mu$ M	50 $\mu$ M	100 $\mu$ M	IC <sub>50</sub>	SI
5	SH-SY5Y	84.09	86.68	84.78	63.24	184.20	
	L929	105.79	101.05	103.62	103.84	-	
6	SH-SY5Y	82.85	93.65	89.06	68.87	250.20	
	L929	93.99	97.21	103.85	101.16	-	
7a	SH-SY5Y	81.90	74.58	63.76	50.63	85.94	9.33
	L929	90.48	88.76	92.13	93.494	801.40	
7b	SH-SY5Y	105.94	95.24	81.21	58.25	188.50	11.7
	L929	98.13	100.06	98.09	95.332	2202	
8a	SH-SY5Y	75.67	76.15	76.34	77.11	189.50	11.6
	L929	95.94	96.45	96.82	97.18	2196	
8b	SH-SY5Y	95.88	97.45	79.42	54.83	165.40	19.1
	L929	96.05	96.48	96.79	97.09	2161	
9a	SH-SY5Y	95.20	96.62	88.87	81.49	455.10	1.76
	L929	85.52	97.95	90.28	92.39	800.10	
9b	SH-SY5Y	79.79	96.35	94.62	57.03	205.20	
	L929	107.89	100.35	135.20	115.88	-	
10	SH-SY5Y	103.81	90.16	107.97	91.97	2116	
	L929	84.19	96.17	92.14	108.30	-	
11	SH-SY5Y	81.59	82.41	63.39	63.77	119.80	1.31
	L929	91.90	78.62	79.35	81.19	260.90	
12	SH-SY5Y	62.79	83.39	73.96	60.58	122.00	2.80
	L929	79.71	92.72	84.71	81.90	341.90	
Vincristine	SH-SY5Y	50.59	48.27	43.53	36.86	25.52	3.37
	L929	70.67	65.80	63.02	61.70	85.94	

**Table 6:** A selection of descriptor values calculated for **5, 7a, 8a, 9a, and 10-12**

Property/ descriptor	Recommended values range	5	7a	8a	9a	10	11	12
#stars	0-5	1	1	2	2	2	2	1
#rtvFG	0-2	1	1	1	1	1	1	1
QPlogPo/w	-2.0 to 6.5	4.53	4.77	4.80	5.05	5.04	5.40	4.99
QPlogS	-6.5 to 0.5	-5.09	-5.38	-5.50	-5.83	-5.86	-6.15	-5.69
QPlogHERG	concern below -5	-7.23	-7.14	-7.13	-7.13	-7.15	-7.00	-7.00
QPPCaco	>500 great	1929	2111	1969	1983	1927	2152	1849
QPlogBB	-3.0 to 1.2	-0.56	-0.43	-0.44	-0.39	-0.40	-0.23	-0.43
QPPMDCK	>500 great	1007	1746	1857	2516	2483	4959	2043
#metab	1 to 8	1	1	1	1	1	1	1
%HOA	>80% is high	100	100	100	100	100	100	100
RuleOfFive	the lower the better	0	0	0	1	1	1	0

**#stars:** Number of descriptors that fall outside the recommended values vary (See additional information for details). **#rtvFG:** Number of functional groups that may cause nonspecific reactions. **QPlogPo/w:** Predicted LogP. **QPlogS:** Predicted water solubility ( $S$  in  $\text{mol}/\text{dm}^3$ ). **QPlogHERG:** Predicted HERG  $K^+$  IC<sub>50</sub>. **QPPCaco:** Predicted permeability in Caco-2 cell model ( $\text{nm}/\text{sec}$ ). **QPlogBB:** Predicted partition coefficient for brain/blood. **QPPMDCK:** Predicted permeability in MDCK cell model ( $\text{nm}/\text{sec}$ ). **#metab:** Number expected metabolic reactions. **%HOA:** Predicted human oral absorption (0 to 100%). **RuleOfFive:** Number of Lipinski's rule of five violations [19]

## DISCUSSION

The title compounds used in this study carry oxime ester derivatives of the ethylene group linking a naphthalene and pyrazole ring. There are several studies in the literature on anticonvulsant, antibacterial, and antifungal activities of derivatives featuring similar skeletons. In addition, the presence of anticarcinogenic activity in numerous heterocyclic compounds containing oxime ester structures have also been investigated in recent

years. A 2001 study on a series of synthetic 2,3,4-trimethoxyacetophenoxime esters containing benzothiazole ring reports antitumor activities through inhibition of extracellular signal-regulated kinase (ERK) phosphorylation in mouse embryonic fibroblast cells (NIH 3T3) (20).

Using human embryonic stem cell line (HSF cell line) to investigate the cytotoxic effects of compounds on healthy cells, another study reports that some of the compounds showed strong cytotoxic effect in comparison to the

reference drug, 5-FU after synthesizing and evaluating a series of 1,4-naphthoquinone oxime-derived compounds for their anticarcinogenic effects on colorectal carcinoma cell lines (21). A similar study synthesizing 1-aryl-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)ethanone derivatives with high yields to monitor their cytotoxic activities against lung (A549), colon (HCT116 and HT29), prostate (DU145) and ovarian (SKOV3) cancer cell lines argues that the naphthalene-containing derivatives were reportedly the most effective compounds against all cell lines. According to the researchers, 1-Naphthyl and 2-naphthyl derivatives were the most effectual compounds in the series while they were also as potent as the standard drug carboplatin against lung (A549) cell lines (22). In the present study, too, we synthesized a series of compounds containing oxime ester and a heterocyclic ring similar to the above-mentioned combinations in order to examine their cytotoxic activities on neuroblastoma cells. In line with the corresponding studies in the literature, our findings show toxicity to neuroblastoma cells at low concentrations. Throughout the tests, we used healthy fibroblast cells to investigate their possible toxic effect and found that they showed selective toxicity to neuroblastoma cells without affecting healthy cells, which proved the therapeutic reliability of our compounds.

Our study differs from the existing studies in its attempt to determine theoretical diastereomer ratio of the oxime components, which has remained a rare research concern in the literature to this day. The compounds converted to their HCl salts were obtained as either *E* isomer or *E/Z* mixtures while those in the parent form were only in *Z* configuration. These results suggest that gHCl has the potential to bring about interconversion in diastereomers possibly due to several factors such as solvation, temperature etc. *E/Z* configuration of each compound was determined according to the chemical shift of the singlet of methylene protons (-CH<sub>2</sub>-N) between the oxime ester group and the pyrazole ring.

Additionally, the compounds were druglike and predicated to have favorable pharmacokinetics according to the *in silico* calculations. Our results demonstrate that the compounds showed favorable pharmacokinetic properties, such as blood-brain partition, aqueous solubility, gut-blood barrier permeability, and oral absorption. Most of the physicochemical and pharmacokinetic properties/descriptors of these compounds were within the limits indicated by the experimental values of known drugs and the compounds were drug-like. On the other hand, a

number of calculations yielded negative results. For example, the solvent accessible surface area values of the compounds fell outside the recommended value range; the oxime ester linker was identified as a nonspecific reactive functional group (#rtvFG); and the compounds were predicted to be potential hERG inhibitors (QPlogHERG). It should also be noted that 9a, 10, and 11 violated Lipinski's Rule of 5 with slightly higher QPlogPo/w values.

Based on the results of our study, it can be suggested that theazole ring and oxime ether structure should be revised in future studies.

## CONCLUSION

The findings of the study show that the cytotoxic effects of the compounds on human neuroblastoma (SH-SY5Y) and fibroblast (L929), indicate that a majority of the compounds exhibit selective cytotoxicity to neuroblastoma cells without significantly affecting fibroblast cell line. The compounds initially had been predicted to be drug-like and to have favorable ADMET properties and this has indeed been confirmed this projection. Thus, more effective compounds can be designed and synthesized through various modifications.

## DECLARATIONS

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### Conflict of interest

No conflict of interest is associated with this work.

### Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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