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Original article

Synthesis of novel 1,2,3-triazole substituted-*N*-alkyl/aryl nitrone derivatives, their anti-inflammatory and anticancer activity

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ABSTRACT

A series of novel 1,2,3-triazole substituted *N*-phenyl nitrone derivatives **5a**–**e** were prepared in three steps starting from 1-substituted-1,2,3-triazole-4-carbaldehydes **2** via Schiff's base formation, reduction followed by oxidation. Similarly, 1,2,3-triazole substituted *N*-alkyl nitrone derivatives **6a**–**p** were prepared in single step starting from compound **2** on reaction with *N*-alkyl hydroxylamine hydrochlorides. All the final compounds were screened for anti-inflammatory and anticancer activity against various cancer cell lines. Among the compounds tested, the compounds **5a**, **5d**, **6a**, **6b**, **6m** and **6o** exhibited significant inhibition of IL-1β secretion as a measure of anti-inflammatory activity. Compound **5b**, **5c**, **6h**, **6i** and **6o** exhibited significant activity against all the cell lines (A549, COLO 205, MDA-MB 231 and PC-3) at IC_{50} values of <15 μM.

1. Introduction

Nitrones are valuable synthons in the realm of organic synthesis which are extensively used in the synthesis of biologically active compounds [1], as spin trap reagents [2], as therapeutic agents [3] and also behave like 1,3-dipoles in cycloaddition reactions [4]. Two main synthetic methods commonly used for the preparation of nitrones are: i) condensation of carbonyl compounds with *N*-mono substituted hydroxylamines [5], ii) oxidation of secondary amines or hydroxylamines [6]. In particular, the oxidative approach provides the most direct and general method for preparing nitrones. Stoichiometric oxidants used in these reactions include oxaziridine [7] or dioxirane [8]. Alternatively, hydrogen peroxide is used as primary oxidant [9] in the presence of a catalyst such as Na_2MoO_4 or Na_2WO_4 [10,11] or $MeReO_3$ [12] or SeO_2 [13]. The 1,2,3-triazoles due to their unique chemical and structural properties have received much attention over the past few decades and found wide application in medicinal chemistry [14–18] and especially as anticancer agents [19–22]. Based on the importance of 1,2,3-triazole, nitrone function and in continuation of our efforts on the synthesis of potential molecules [23–26], we have synthesized a series of novel 1,2,3-triazole substituted-*N*-alkyl/aryl nitrone derivatives and are presented herein for the first time. In this communication, *N*-((1-substituted-1*H*-1,2,3-triazol-4-yl)methyl) anilines **4** are oxidized into the corresponding nitrones **5** upon treatment with 2–3 molar equivalents of hydrogen peroxide in the presence of 4–5 mol % of selenium dioxide at room temperature in a single step. The synthesis of 1,2,3-triazole substituted-*N*-alkyl nitrone derivatives **6** was accomplished by condensation of 1-substituted-(1,2,3)triazole-4-carbaldehydes **2** [27] with *N*-mono substituted hydroxylamine hydrochlorides.

2. Chemistry

The 1-substituted-1,2,3-triazol-4-yl-methanol **1** was oxidized to an aldehyde **2** using Jones reagent followed by the reaction of aniline in acetonitrile at room temperature furnished Schiff's base **3**. The *N*-((1-alkyl-1*H*-1,2,3-triazol-4-yl) methylene) aniline **3** were reduced to amine **4** using $NaBH_4$ in methanol and the resulting reduced product further oxidized with 2–3 molar equivalents of hydrogen peroxide and 4–5 mol% of selenium dioxide to obtain

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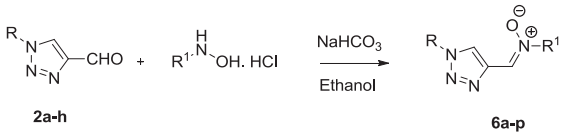
1,2,3-triazole substituted-*N*-phenyl nitrone derivatives **5a**–**e**. The activity of various oxidants such as t-BuOOH, m-$ClC_6H_4CO_3H$ and H_2O_2 have been examined and amongst them hydrogen peroxide was identified as the best oxidant. The reaction could proceed efficiently with H_2O_2 and SeO_2 system in DCM medium. The details of the reactions are outlined in Scheme 1. Similarly, 1-phenyl-1,2,3-triazole-4-carbaldehyde **2a** was reacted with *N*-benzyl hydroxylamine hydrochloride using different bases such as Et_3N, piperidine, K_2CO_3 and $NaHCO_3$ in ethanol and obtained 1,2,3-triazole substituted *N*-benzyl nitrone derivative **6a** in a single step. Among all the bases, $NaHCO_3$ is considered as best which gave the highest yield. The protocol was extended with diverse substituted 1,2,3-triazole-4-carbaldehydes with *N*-benzyl/*t*-butyl substituted hydroxylamine hydrochlorides and obtained respective products in high yield. The reactions are outlined in Scheme 2 and products were tabulated in Table 1.



R= C_6H_5, 4-FC_6H_4, 4-$CF_3C_6H_4$, 4-ClC_6H_4, 4-$MeOC_6H_4$, $C_6H_{13}CH_2CH_2$, $C_6F_{13}CH_2CH_2$, $C_8H_{17}CH_2CH_2$, $C_8F_{17}CH_2CH_2$.
R^1 = Benzyl, *t*-Butyl

Scheme 2. Synthesis of 1,2,3-triazole substituted-*N*-alkyl nitrone derivatives **6a**–**p**.

Table 1
Physical properties of compounds **5a**–**e** and **6a**–**p**.

S. No	Compound no	R	R^1	Yield (%)[a]	m.p (°C)
1	**5a**	C_6H_5	C_6H_5	64	126–128
2	**5b**	4-FC_6H_4	C_6H_5	62	132–134
3	**5c**	4-ClC_6H_4	C_6H_5	61	135–136
4	**5d**	$C_8H_{17}CH_2CH_2$	C_6H_5	58	124–125
5	**5e**	$C_8F_{17}CH_2CH_2$	C_6H_5	54	142–144
6	**6a**	C_6H_5	Benzyl	88	150–152
7	**6b**	4-FC_6H_4	Benzyl	85	160–162
8	**6c**	4-$MeOC_6H_4$	Benzyl	84	134–136
9	**6d**	4-$CF_3C_6H_4$	Benzyl	88	174–176
10	**6e**	$C_6H_{13}CH_2CH_2$	Benzyl	79	142–144
11	**6f**	$C_8H_{17}CH_2CH_2$	Benzyl	82	148–150
12	**6g**	$C_6F_{13}CH_2CH_2$	Benzyl	74	180–182
13	**6h**	$C_8F_{17}CH_2CH_2$	Benzyl	77	194–196
14	**6i**	C_6H_5	*t*-Butyl	89	144–146
15	**6j**	4-FC_6H_4	*t*-Butyl	85	145–146
16	**6k**	4-$MeOC_6H_4$	*t*-Butyl	84	108–110
17	**6l**	4-$CF_3C_6H_4$	*t*-Butyl	88	156–158
18	**6m**	$C_6H_{13}CH_2CH_2$	*t*-Butyl	82	76–78
19	**6n**	$C_8H_{17}CH_2CH_2$	*t*-Butyl	85	82–84
20	**6o**	$C_6F_{13}CH_2CH_2$	*t*-Butyl	71	140–142
21	**6p**	$C_8F_{17}CH_2CH_2$	*t*-Butyl	74	164–166

[a] Isolated yields.

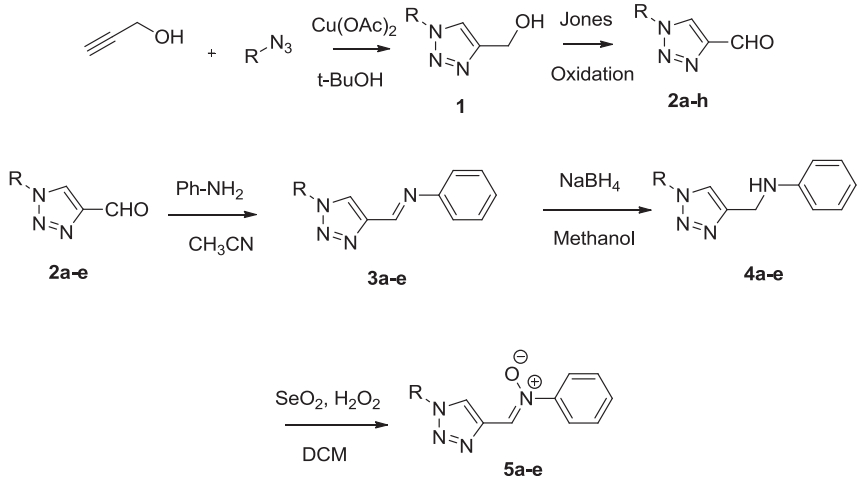
3. Results and discussion

3.1. Anti-inflammatory activity

All the final compounds **5a**–**e** and **6a**–**p** were screened for anti-inflammatory activity in the presence of PMA (phorbol 13-myristate 12-acetate)-induced inflammation using THP-1 monocyte cell system [31]. Among the compounds tested, the compounds **5a**, **5d**, **6a**, **6b**, **6m** and **6o** exhibited significant inhibition of IL-1β secretion as a measure of anti-inflammatory activity (IC_{50} 7.9–9.9 μM, Pl. see Table 2). Under the same conditions, piroxicam, a well known COX-2 inhibitor showed an IC_{50} of 18 μM (Table 2). On the other hand, none of the compounds even at 20 μM concentration had any effect on cell viability (data not shown). This clearly indicates that the anti-inflammatory activities of these compounds are not due to the induction of cytotoxicity in these cells. The structure–activity relationship (SAR) revealed that the activity is independent of structure and there was no clear cut relationship. Further studies are underway to optimize the lead molecule.

3.2. In vitro cytotoxicity

The compounds **5a**–**e** and **6a**–**p** were also screened against four human cancer cell lines such as A549, COLO 205, MDA-MB 231 and PC-3 by MTT assay method [32]. IC_{50} values of the test compounds on different cancer cell lines were calculated and the results are presented in Table 3. Based on the results, it is evident that most of the compounds showed significant reduction in cell viability in all the tested cell lines in a concentration-dependent manner. Among the derivatives, compound **5b**, **5c**, **6h**, **6i** and **6o** exhibited significant activity against all the cell lines at micro molar concentration with IC_{50} values of <15 μM. Compounds **6k**, **6l**, **6m**, **6n** and **6p** exhibited moderate activity against all the cell lines at IC_{50} values of <50 μM. Similarly, the compound **6j** showed moderate activity on all the cell lines at IC_{50} value of <90 μM and compound **5e** showed cytotoxicity against A549 cell line only at IC_{50} value of <86.8 μM.



Scheme 1. Synthesis of 1,2,3-triazole substituted-*N*-phenyl nitrone derivatives **5a**–**e**.

Table 2
IL-1β secretion inhibition efficacy of the synthesized compounds **5a**–**e** and **6a**–**p**.[a]

S. No	Compounds	IL-1β (IC_{50} μM)[b]
1	**5a**	8.8 ± 1.6
2	**5b**	NA
3	**5c**	NA
4	**5d**	9.9 ± 2.3
5	**5e**	11.7 ± 1.6
6	**6a**	8 ± 2.6
7	**6b**	9.7 ± 3.5
8	**6c**	17.3 ± 1.6
9	**6d**	15.4 ± 3.2
10	**6e**	13.1 ± 2.6
11	**6f**	15.6 ± 0.8
12	**6g**	12.1 ± 3.1
13	**6h**	17.0 ± 2.6
14	**6i**	NA
15	**6j**	14 ± 1.1
16	**6k**	13.5 ± 2.8
17	**6l**	14.4 ± 1.0
18	**6m**	7.9 ± 1.36
19	**6n**	20.0 ± 1.67
20	**6o**	8.1 ± 2.01
21	**6p**	15.0 ± 3.7
22	Piroxicam[c]	18.0 ± 2.7

[a] THP1 monocytes were pre-treated with 5, 10 and 20 μM concentrations of the above mentioned 1,2,3-triazole substituted nitrone derivatives **5a**–**e** and **6a**–**p** for 2 h before simulation with 100 nM Phorbol 13-myristate 12-acetate (PMA) to induce inflammation for a period of 48 h. At the end of the treatment, conditioned media was collected and the level of IL-1β was measured by ELISA as described in the experimental section.

[b] IC_{50} values are mean ± SD of three independent experiments, NA: indicates IC_{50} value >20 μM.

[c] Piroxicam, a known anti-inflammatory agent was used as a positive control.

The structure–activity relationship (SAR) studies revealed that 4-fluorophenyl or 4-chlorophenyl substitution on the triazole moiety and phenyl, t-butyl on nitrone moiety promoted the cytotoxicity, whereas the benzyl group substituent on the nitrone moiety exhibited no additional advantage in improving the cytotoxicity. Among all the derivatives, compounds **5b**, **5c**, **6h** and **6o** showed potent cytotoxicity against all the tested cell lines at IC_{50} values of <12 μM. However, compounds **6a**–**g** did not show any cytotoxicity. Further, it was confirmed that the promising compound **6h** exhibited apoptotic cell death based on the caspase-3 assay (Table 4). Nevertheless, it is contemplated that a slight structural modification of these active derivatives may yield prospective anticancer drugs.

4. Experimental section

Melting points of all the compounds was recorded on Casia-Siamia (VMP-AM) melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer FT-IR 240-C spectrophotometer using KBr optics. ^1H NMR spectra were recorded on Bruker AV 300 MHz and INOVA 500 MHz in $CDCl_3$, ^{13}C NMR spectra were recorded on Bruker AV 75 MHz in $CDCl_3$ & DMSO-d_6 using TMS as an internal standard. CHN analysis was recorded on a Vario EL analyser. All high-resolution spectra were recorded on QSTARXL hybrid MS/MS system (Applied Bio systems, USA) under electro spray ionization. All the reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel 60 F_{254} (mesh); spots were visualized with UV light. Merck silica gel (60–120 mesh) was used for column chromatography. All the final compounds were analyzed by HPLC to evaluate purity using SHIMADZU LC-20 instrument. Separation was carried out by using a mobile phase of ammonium acetate: acetonitrile (40:60) on Zodiac C_{18} Column (150 mm × 4.6 mm, 5 μM) in an isocratic mode at a flow rate of 1.0 ml/min with Photo-diode Array (PDA) detection at 254 nm.

4.1. Preparation of alkyl/aryl azides [23]

4.1.1. General procedure

Iodo alkane (10.0 mmol) and sodium azide (30.0 mmol) were taken in 40 mL acetone: water (3:1) and heated to 60 °C for 8 h. After completion of reaction, cooled to room temperature and excess solvent was removed under vacuum. The aqueous residue was extracted with hexane (3×, 30 mL each time) and the combined extracts were washed with water, then dried over anhydrous sodium sulfate. The solvent was removed under vacuum to obtain alkyl azide in quantitative yield and used in the next reaction without further purification.

4.2. Preparation of 1-substituted-1,2,3-triazol-4-yl-methanol (*1*)

4.2.1. General procedure

In an argon atmosphere, alkyl/aryl azide (5.4 mmol), propargyl alcohol (8 mmol) were taken in *t*-BuOH (5 mL) followed by addition of an aqueous $Cu(OAc)_2$ solution (5 mol%, 1 mL). The reaction was stirred for 18 h at room temperature and monitored by TLC. The resulting mixture was diluted with dichloromethane; the organic layer was separated and washed with water. Dried over sodium sulfate and purified by passing through a column packed with silica gel using n-hexane: ethyl acetate (6:4) as solvents.

(1-Phenyl-1H-(1,2,3)triazol-4-yl)-methanol (**1a**) [28]
(1-(4-Fluoro-phenyl)-1H-(1,2,3)triazol-4-yl)-methanol (**1b**) [23]
(1-(4-Chlorophenyl)-1H-(1,2,3)triazol-4-yl)-methanol (**1c**) [29]
(1-decyl-1H-(1,2,3)triazol-4-yl)-methanol(**1d**) [23]
(1-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptadecafluoro-decyl)-1H-(1,2,3)triazol-4-yl)-methanol (**1e**) [19].

4.3. Preparation of 1-substituted-1,2,3-triazol-4-carbaldehyde (*2*)

4.3.1. General procedure

The 1-substituted-1,2,3-triazol-4-yl-methanol **1** (4 mmol) was taken in dry acetone (10 mL), cooled to 0 °C and the Jones reagent (CrO_3 + H_2SO_4 + Acetone) (4 mmol) was added slowly over a period of 15 min. The reaction mixture was stirred for 20 min at 0 °C. After completion of reaction, filtered through the short pad of celite and the filtrate was collected and concentrated under vacuum. The residue was purified by passing through a column packed with silica gel using petroleum ether/EtOAc (8:2) as eluents.

1-Phenyl-1H-(1,2,3)triazol-4-carbaldehyde (**2a**) [30]
1-(4-Fluorophenyl)-1H-(1,2,3)triazol-4-carbaldehyde (**2b**) [23]
1-(4-Chlorophenyl)-1H-(1,2,3)triazol-4-carbaldehyde (**2c**) [29]
1-Decyl-1H-(1,2,3)triazol-4-carbaldehyde (***2d***) [23]
1-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptadecafluoro-decyl)-1H-(1,2,3)triazole-4-carbaldehyde (***2e***) [19].

4.4. Preparation of N-((1-alkyl-1H-1,2,3-triazol-4-yl) methylene) aniline (*3*)

4.4.1. General procedure

The 1-substituted-1,2,3-triazol-4-carbaldehyde **2** (4 mmol) and aniline (4 mmol) were taken in acetonitrile (10 mL). The reaction mixture was stirred at room temperature for 6 h and after completion of reaction, as monitored by TLC, acetonitrile was removed under vacuum. The residue was purified by passing through a column packed with silica gel using petroleum ether/EtOAc (8:2) as eluents.

Table 3
Anticancer activity of compounds **5a**–**e** and **6a**–**p**.[a] IC_{50}[b] values in μM.

S. No	Compound	A549	COLO 205	MDA-MB 231	PC-3
1	**5a**	–	–	–	–
2	**5b**	12.5 ± 0.22	10.2 ± 0.27	9.8 ± 0.09	11.4 ± 0.32
3	**5c**	10.5 ± 0.24	9.7 ± 0.42	8.9 ± 0.43	11.1 ± 0.36
4	**5d**	–	–	–	–
5	**5e**	86.8 ± 0.42	–	–	
6	**6a**	–	–	–	
7	**6b**	–	–	–	–
8	**6c**	–	–	–	–
9	**6d**	–	–	–	–
10	**6e**	–	–	–	–
11	**6f**	–	–	–	–
12	**6g**	–	–	–	–
13	**6h**	8.8 ± 0.19	9.4 ± 0.18	8.4 ± 0.33	8.1 ± 0.32
14	**6i**	17.4 ± 0.17	15.2 ± 0.15	14.8 ± 0.28	15.4 ± 0.34
15	**6j**	87.0 ± 0.32	58.9 ± 0.48	66.4 ± 0.22	72.1 ± 0.52
16	**6k**	23.7 ± 0.44	19.8 ± 0.27	23.1 ± 0.09	23.8 ± 0.28
17	**6l**	43.6 ± 0.35	44.7 ± 0.38	48.2 ± 0.15	42.0 ± 0.18
18	**6m**	31.3 ± 0.25	30.8 ± 0.52	29.7 ± 0.26	24.6 ± 0.11
19	**6n**	24.8 ± 0.42	22.1 ± 0.25	20.8 ± 0.22	19.8 ± 0.08
20	**6o**	11.5 ± 0.41	9.5 ± 0.34	9.8 ± 0.32	11.2 ± 0.22
21	**6p**	30.5 ± 0.15	28.8 ± 0.28	29.7 ± 0.38	25.7 ± 0.18
22	Doxorubicin[c]	0.8 ± 0.22	0.7 ± 0.42	0.8 ± 0.44	0.6 ± 0.28

[a] Exponentially growing cells were treated with different concentrations of 1,2,3-triazole substituted-*N*-phenyl nitrone derivatives **5a**–**e** and 1,2,3-triazole substituted-*N*-alkyl nitrone derivatives **6a**–**p** for 24 h and cell growth inhibition was analyzed through MTT assay.
[b] IC_{50} is defined as the concentration, which results in a 50% decrease in cell number as compared with that of the control cultures in the absence of an inhibitor and were calculated from the plotted absorbance data for the dose–response curves. The values (in μM) represent the mean ± SE of three independent experiments.
[c] Doxorubicin was employed as positive control, – indicates IC_{50} value >100 μM.

*N-((1-Phenyl-1H-1,2,3-triazol-4-yl)methylene)aniline (**3a**)*

White solid, Yield: 91%, m.p.: 110–112 °C, IR (KBr, cm^{-1}): 3018 (HC═C), 1532 (–C═N); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.16–7.24 (m, 5H, Ar-H), 7.32–7.36 (m, 3H, Ar-H), 7.80 (d, 2H, *J* = 7.8 Hz, Ar-H), 8.42 (s, 1H, Ar-H), 8.71 (s, 1H, –CH═N); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 151.8, 151.1, 147.2, 136.7, 130.5, 128.3, 127.2, 122.7, 121.6, 120.4; MS (ESI, 70 eV): 249 *m*/*z* (M + H); Anal. Calcd. for $C_{15}H_{12}N_4$: C, 72.56; H, 4.87; N, 22.57%: Found: C, 72.54; H, 4.89; N, 22.61%.

*N-((1-(4-Fluorophenyl)-1H-1,2,3-triazol-4-yl)methylene)aniline (**3b**)* [23]
*N-((1-(4-Chlorophenyl)-1H-1,2,3-triazol-4-yl)methylene)aniline (**3c**)*

White solid, Yield: 94%, m.p.: 132–134 °C, IR (KBr, cm^{-1}): 3012 (HC═C), 1554 (–C═N); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.12–7.21 (m, 5H, Ar-H), 7.26 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.68 (d, 2H, *J* = 8.1 Hz, Ar-H), 8.43 (s, 1H, Ar-H), 8.73 (s, 1H, –CH═N); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 151.7, 151.2, 147.0, 134.9, 134.3, 130.0, 128.8, 127.2, 122.3, 121.9, 121.4; MS (ESI, 70 eV): 283 *m*/*z* (M + H); Anal. Calcd. for $C_{15}H_{11}ClN_4$: C, 63.72; H, 3.92; N, 19.82%: Found: C, 63.74; H, 3.91; N, 19.85%.

*N-((1-Decyl-1H-1,2,3-triazol-4-yl)methylene)aniline (**3d**)* [23]
*N-((1-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptadecafluorodecyl)-1H-1,2,3-triazol-4-yl)methylene)aniline (**3e**)* [19]

4.5. Preparation of N-((1-substituted-1H-1,2,3-triazol-4-yl)methyl) aniline (*4*)

4.5.1. General procedure

A solution of *N*-((1-alkyl-1*H*-1,2,3-triazol-4-yl)methylene)aniline **3** (4.7 mmol) in MeOH (10 mL) was cooled at 0 °C. Then $NaBH_4$ (9.4 mmol) was added slowly and the final mixture was stirred during 2 h at room temperature. After completion of the reaction, solvent was evaporated *in vacuo* and the residue was treated with H_2O (50.0 mL). Extracted with EtOAc (3 × 10.0 mL), combined ethyl acetate layer was dried over anhydrous $NaSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography [SiO_2, petroleum ether/EtOAc (7:3)].

N-((1-Phenyl-1H-1,2,3-triazol-4-yl)methyl)aniline (**4a**)

White solid, Yield: 76%, m.p.: 102–104 °C, IR (KBr, cm^{-1}): 3340 (–NH), 2988 (HC═C), 1521 (–C═C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.51 (s, 2H, $–CH_2–$N), 4.62 (br.s, 1H, NH), 6.63–6.74 (m, 3H, Ar-H), 7.12 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.19 (m, 3H, Ar-H), 7.59 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.85 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 149.1, 136.8, 132.2, 129.7, 128.5, 120.8, 120.1, 119.2, 112.7, 42.5; MS (ESI, 70 eV): 251 *m*/*z* (M + H); Anal. Calcd. for $C_{15}H_{14}N_4$: C, 71.98; H, 5.64; N, 22.38%. Found: C, 71.99; H, 5.66; N, 22.42%.

N-((1-(4-Fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)aniline (**4b**)

White solid, Yield: 69%, m.p.: 128–129 °C, IR (KBr, cm^{-1}): 3328 (–NH), 2972 (HC═C), 1531 (–C═C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.55 (s, 2H, $–CH_2–$N), 4.32 (br.s, 1H, NH), 6.69–6.79 (m, 3H, Ar-H), 7.18 (d, *J* = 7.3 Hz, 2H, Ar-H), 7.22 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.68 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.83 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 160.2, 147.1, 133.8, 132.2, 129.4, 127.5, 121.8, 120.4, 119.1, 112.7, 42.3; MS (ESI, 70 eV): 269 *m*/*z* (M + H); Anal. Calcd. for $C_{15}H_{13}FN_4$: C, 67.15; H, 4.88; N, 20.88%. Found: C, 67.18; H, 4.86; N, 20.89%.

N-((1-(4-Chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)aniline (**4c**)

White solid, Yield: 72%, m.p.: 126–128 °C, I.R. (KBr, cm^{-1}): 3309 (–NH), 2978 (HC═C), 1511 (–C═C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.61 (s, 2H, $–CH_2–$N), 4.54 (br.s, 1H, NH), 6.71–6.80 (m, 3H, Ar-H), 7.21 (d, *J* = 7.24 Hz, 2H, Ar-H), 7.28 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.81 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.86 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 154.2, 147.4, 134.8, 132.8, 128.4, 126.5, 120.8, 120.4, 118.1, 112.2, 42.5; MS (ESI, 70 eV): 283 *m*/*z* (M + H); Anal. Calcd. for

Table 4
Caspase-3 activity on compound **6h**.

S. No.	Test compound (concentration)	Fluorescence units (FU/mg/min)			
		12 h	24 h	36 h	48 h
1	Control	1055.6 ± 108.2	2115.3 ± 83.2	2192.3 ± 115.9	2642.4 ± 37.5
2	**6h** (8 μM)	2473.2 ± 104.1	3148.5 ± 281.9	3396.8 ± 209.1	4280.8 ± 170.8
3	**6h** (10 μM)	2541.4 ± 125.1	3802.2 ± 182.9	4082.5 ± 176.6	4700.5 ± 28.9
4	Doxorubicin (1 μM)	3033.7 ± 136.8	3791.8 ± 327.0	4794.2 ± 152.1	6690.3 ± 173.7
5	Doxorubicin (2 μM)	3331.1 ± 356.8	4007.5 ± 213.5	4994.1 ± 149.2	7240.4 ± 271.9

$C_{15}H_{13}ClN_4$: C, 63.27; H, 4.60; N, 19.68%. Found: C, 63.29; H, 4.61; N, 19.62.

N-((1-Decyl-1H-1,2,3-triazol-4-yl)methyl)aniline (**4d**)

White solid, Yield: 79%, m.p.: 98–99 °C, IR (KBr, cm^{-1}): 3324 (−NH), 2945 (HC═C), 1518 (−C═C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.88 (t, J = 7.05 Hz, 3H, $-CH_3$), 1.23–1.33 (m, 14H, $-(CH_2)_7-$), 1.84–1.90 (m, 2H, $-CH_2$), 4.20 (br.s, 1H, −NH), 4.30 (t, J = 6.9 Hz, 2H, $-NCH_2$), 4.45 (s, 2H, $-CH_2-NH$), 6.67 (d, J = 7.1 Hz, 2H, Ar-H), 6.74 (t, J = 7.1 Hz, 1H, Ar-H), 7.18 (t, J = 8.05 Hz, 2H, Ar-H), 7.41 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 149.3, 130.2, 129.5, 122.7, 120.8, 112.2, 52.4, 42.5, 31.9, 29.6, 28.4, 27.1, 22.7, 14.2; MS (ESI, 70 eV): 315 m/z (M + H); Anal. Calcd. for $C_{19}H_{30}N_4$: C, 72.57; H, 9.62; N, 17.82%. Found: C, 72.54; H, 9.63; N, 17.85%.

N-((1-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptadecafluorodecyl)-1H-1,2,3-triazol-4-yl)methyl)aniline (**4e**)

White solid, Yield: 62%, m.p.: 122–124 °C, IR (KBr, cm^{-1}): 3314 (−NH), 2945 (HC═C), 1518 (−C═C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.82–2.91 (m, 2H, $-CH_2$), 4.44 (t, J = 6.94 Hz, 2H, $-NCH_2$), 4.67 (s, 2H, $-CH_2-NH$), 4.78 (br.s, 1H, NH), 6.80–6.89 (m, 3H, Ar-H), 7.32 (t, J = 8.15 Hz, 2H, Ar-H), 7.88 (s, 1H, Ar-H); MS (ESI, 70 eV): 621 m/z (M + H); Anal. Calcd. for $C_{19}H_{13}F_{17}N_4$: C, 36.79; H, 2.11; N, 9.03%. Found: C, 36.81; H, 2.14; N, 9.10%.

4.6. Preparation of N-((1-substituted-1H-1,2,3-triazol-4-yl) methylene)aniline oxide (**5**)

4.6.1. General procedure

To a mixture of SeO_2 (15 mol%) and *N*-((1-substituted-1*H*-1,2,3-triazol-4-yl)methyl)aniline **4** (2.59 mmol) in DCM (5.0 mL) was added drop wise an aqueous 30% hydrogen peroxide solution (7.56 mmol) at 0 °C under argon and stirred at room temperature for 3 h. After completion of reaction as indicated by TLC, DCM was removed under reduced pressure and the residue was purified by passing through a column packed with silica gel using petroleum ether/EtOAc (7:3) as eluents.

N-((1-Phenyl-1H-1,2,3-triazol-4-yl)methylene)aniline oxide (**5a**)

White solid, Purity: 97.2%; Yield: 64%, m.p.: 126–128 °C, IR (KBr, cm^{-1}): 2915 (HC═C), 1565 (−C═N); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.28 (t, J = 8.12 Hz, 2H, Ar-H), 7.49–7.58 (m, 5H, Ar-H), 7.80–7.88 (m, 3H, Ar-H), 8.61 (s, 1H, Ar-H), 9.48 (s, 1H, HC═N); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 139.9, 136.3, 132.0, 129.5, 129.3, 129.0, 127.0, 123.5, 120.2; MS (ESI, 70 eV): 265 m/z (M + H); Anal. Calcd. for $C_{15}H_{12}N_4O$: C, 68.17; H, 4.58; N, 21.20%. Found: C, 68.15; H, 4.57; N, 21.24%.

N-((1-(4-Fluorophenyl)-1H-1,2,3-triazol-4-yl)methylene)aniline oxide (**5b**)

White solid, Purity: 97.8%; Yield: 62%, m.p.: 132–134 °C, IR. (KBr, cm^{-1}): 2967 (HC═C), 1556 (−C═N); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.31 (t, J = 8.8 Hz, 2H, Ar-H), 7.51–7.59 (m, 4H, Ar-H), 7.89 (m, 3H, Ar-H), 8.62 (s, 1H, Ar-H), 9.50 (s, 1H, HC═N); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 162.0, 141.2, 139.3, 130.9, 130.0, 127, 124.6, 123.5, 122.7, 122.3, 120.3; MS (ESI, 70 eV): 283 m/z (M + H); Anal. Calcd. for $C_{15}H_{11}FN_4O$: C, 63.83; H, 3.93; N, 19.85%. Found: C, 63.86; H, 3.94; N, 19.86%.

N-((1-(4-Chlorophenyl)-1H-1,2,3-triazol-4-yl)methylene)aniline oxide (**5c**)

White solid, Purity: 98.1%; Yield: 61%, m.p.: 135–136 °C, IR. (KBr, cm^{-1}): 2911 (HC═C), 1571 (−C═N); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.39 (d, J = 7.49 Hz, 2H, Ar-H), 7.45–7.52 (m, 3H, Ar-H), 7.52 (d, J = 7.49 Hz, 2H, Ar-H), 7.64 (d, J = 7.54 Hz, 2H, Ar-H), 8.08 (s, 1H, Ar-H), 9.11 (s, 1H, HC═N); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 142.2, 134.1, 132.8, 132.2, 128.7, 127.5, 127.03, 125.2, 123.7, 122.4, 115.5; MS (ESI, 70 eV): 299 m/z (M + H); Anal. Calcd. for $C_{15}H_{11}ClN_4O$: C, 60.31; H, 3.71; N, 18.76%. Found: C, 60.36; H, 3.72; N, 18.75%.

N-((1-Decyl-1H-1,2,3-triazol-4-yl)methylene)aniline oxide (**5d**)

White solid, Purity: 98.4%; Yield: 58%, m.p.: 124–125 °C, IR (KBr, cm^{-1}): 2987 (HC═C), 1544 (−C═N); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.88 (t, J = 6.98 Hz, 3H, CH_3), 1.24–1.36 (m, 14H, $-(CH_2)_2-$), 1.91–2.01 (m, 2H, CH_2), 4.44 (t, J = 6.42 Hz, 2H, CH_2), 7.41–7.46 (m, 3H, Ar-H), 7.54 (d, J = 8.1 Hz, 2H, Ar-H), 8.10 (s, 1H, Ar-H), 9.15 (s, 1H, HC═N); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 139.3, 132.1, 129.3, 128.9, 128.7, 127.5, 125.3, 50.3, 31.6, 29.8, 28.1, 22.4, 13.8; MS (ESI, 70 eV): 329 m/z (M + H); Anal. Calcd. for $C_{19}H_{28}N_4O$: C, 69.48; H, 8.59; N, 17.06%: Found: C, 69.52; H, 8.56; N, 17.09%.

N-((1-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptadecafluorodecyl)-1H-1,2,3-triazol-4-yl)methylene)aniline oxide (**5e**)

White solid, Purity: 97.6%; Yield: 54%, m.p.: 142–144 °C, IR (KBr, cm^{-1}): 2921 (HC═C), 1593 (−C═N); ^{1}H NMR (DMSO-d_6, 300 MHz): 2.84–2.95 (m, 2H, $-CH_2$), 4.72 (t, J = 7.2 Hz, 2H, CH_2), 7.24 (t, J = 7.6 Hz, 3H, Ar-H), 7.45 (d, J = 7.7 Hz, 2H, Ar-H), 8.18 (s, 1H, Ar-H), 9.89 (s, 1H, −CH═N); MS (ESI, 70 eV): 635 m/z (M + H); Anal. Calcd. for $C_{19}H_{11}F_{17}N_4O$: C, 35.98; H, 1.75; N, 8.83%: Found: C, 35.99; H, 1.74; N, 8.87%.

4.7. Synthesis of 1,2,3-triazole substituted-N-alkyl nitrone derivatives (**6a–p**)

4.7.1. General procedure

A mixture of 1-substituted-1*H*-1,2,3-triazole-4-carbaldehyde **2** (1 mmol), *N*-benzyl/t-butyl hydroxylamine hydrochloride (1.5 mmol) and sodium bicarbonate (1.5 mmol) in absolute ethanol (6 mL) was heated at 60 °C until the carbonyl compound was disappeared (monitored by TLC). The solvent was removed *in vacuo* and the reaction mixture was diluted with H_2O and extracted with EtOAc. Combined organic layers dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography (SiO_2, mixtures of petroleum ether/EtOAc).

1-Phenyl-N-((1-phenyl-1H-1,2,3-triazol-4-yl)methylene)methanamine oxide (**6a**)

White solid, Purity: 98.8%; Yield: 88%, m.p.: 150–152 °C, IR (KBr, cm^{-1}): 2957 (HC═C), 1598 (−C═N); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.11 (s, 2H, CH_2N), 7.42–7.47 (m, 3H, Ar-H), 7.48–7.52 (m, 3H, Ar-H), 7.54 (t, J = 6.79 Hz, 2H, Ar-H), 7.78 (d, J = 8.3 Hz, 2H, Ar-H), 7.92 (s, 1H, Ar-H), 8.30 (s, 1H, HC═N); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 140.1, 136.5, 132.2, 129.7, 129.5, 129.03, 127.2, 123.7, 120.4, 69.6; MS (ESI, 70 eV): 279 m/z (M + H); HRMS m/z calcd. for $C_{16}H_{15}ON_4$ [M + H]: 279.1240. Found 279.1233.

N-((1-(4-Fluorophenyl)-1H-1,2,3-triazol-4-yl)methylene)-1-phenylmethanamine oxide (**6b**)

White solid, Purity: 98.5%; Yield: 85%, m.p.: 160–162 °C, IR. (KBr, cm^{-1}): 2987 (HC=C), 1541 (−C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 5.12 (s, 2H, CH_2N), 7.25 (t, J = 8.9 Hz, 2H, Ar-H), 7.43 (m, 3H, Ar-H), 7.50 (d, J = 7.9 Hz, 2H, Ar-H), 7.78 (d, J = 7.9 Hz, 2H, Ar-H), 8.04 (s, 1H, Ar-H), 9.27 (s, 1H, HC=N); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 162.2, 141.3, 139.1, 130.9, 130.6, 127.1, 124.5, 123.1, 122.7, 122.3, 120.2, 70.3; MS (ESI, 70 eV): 297 m/z (M + H); HRMS m/z calcd. for $C_{16}H_{14}ON_4F$ [M + H]: 297.1146. Found 297.1139.

N-((1-(4-Methoxyphenyl)-1H-1,2,3-triazol-4-yl)methylene)-1-phenylmethanamine oxide (**6c**)

White solid, Purity: 98.8%; Yield: 84%, m.p.: 134–136 °C, IR (KBr, cm^{-1}): 2978 (HC=C), 1576 (−C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 3.87 (s, 3H, OCH_3), 5.10 (s, 2H, CH_2N), 7.02 (d, J = 8.8 Hz, 2H, Ar-H), 7.40–7.51 (m, 5H, Ar-H), 7.66 (d, J = 8.8 Hz, 2H, Ar-H), 7.90 (s, 1H, Ar-H), 9.21 (s, 1H, HC=N); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 159.8, 139.8, 132.5, 129.9, 129.5, 129.2, 129.0, 127.4, 123.4, 122.0, 114.5, 69.5, 55.5; MS (ESI, 70 eV): 331 m/z (M + Na); HRMS m/z calcd. for $C_{17}H_{17}O_2N_4$ [M + H]: 309.1346. Found 309.1336.

1-Phenyl-N-((1-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl) methylene)methanamine oxide (**6d**)

White solid, Purity: 99.2%; Yield: 88%, m.p.: 174–176 °C, IR (KBr, cm^{-1}): 2986 (HC=C), 1541 (−C=N), 1292 (C–F); ^1H NMR (DMSO-d_6, 300 MHz): δ 5.12 (s, 2H, CH_2N), 7.43–7.51 (m, 5H, Ar-H), 7.82 (d, J = 8.3 Hz, 2H, Ar-H), 7.92 (d, J = 8.3 Hz, 2H, Ar-H), 7.96 (s, 1H, Ar-H), 9.38 (s, 1H, HC=N); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 140.4, 135.5, 134.2, 132.7, 131.5, 128.3, 127.7, 127.1, 125.4, 124.2, 123.6, 123.1, 65.6; MS (ESI, 70 eV): 369 m/z (M + Na); HRMS m/z calcd. for $C_{17}H_{14}ON_4F_3$ [M + H]: 347.1114. Found 347.1107.

N-((1-Octyl-1H-1,2,3-triazol-4-yl)methylene)-1-phenylmethanamine oxide (**6e**)

White solid, Purity: 99.2%; Yield: 79%, m.p.: 142–144 °C, IR (KBr, cm^{-1}): 2957 (HC=C), 1588 (−C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 0.86 (t, J = 6.7 Hz, 3H, CH_3), 1.21–1.34 (m, 10H, $-(CH_2)_5-$), 1.85–1.96 (m, 2H, CH_2), 4.38 (t, J = 7.5 Hz, 2H, CH_2), 5.07 (s, 2H, CH_2N), 7.39–7.49 (m, 5H, Ar-H), 7.83 (s, 1H, Ar-H), 8.81 (s, 1H, HC=N); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 139.5, 132.3, 129.5, 129.3, 129.0, 127.6, 125.5, 69.4, 50.4, 31.6, 30.0, 28.8, 26.3, 22.5, 13.9; MS (ESI, 70 eV): 315 m/z (M + H); HRMS m/z calcd. for $C_{18}H_{27}ON_4$ [M + H]: 315.2179. Found 315.2171.

N-((1-Decyl-1H-1,2,3-triazol-4-yl)methylene)-1-phenylmethanamine oxide (**6f**)

White solid, Purity: 98.1%; Yield: 82%, m.p.: 148–150 °C, IR (KBr, cm^{-1}): 2892 (HC=C), 1581 (−C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 0.87 (t, J = 6.04 Hz, 3H, CH_3), 1.22–1.34 (m, 14H, $-(CH_2)_7-$), 1.86–1.96 (m, 2H, CH_2), 4.37 (t, J = 6.8 Hz, 2H, CH_2), 5.06 (s, 2H, CH_2N), 7.40–7.48 (m, 5H, Ar-H), 7.83 (s, 1H, Ar-H), 8.81 (s, 1H, HC=N); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 139.5, 132.3, 129.5, 129.2, 129.0, 127.7, 125.5, 69.5, 50.5, 31.8, 30.0, 28.3, 22.6, 14.0; MS (ESI, 70 eV): 343 m/z (M + H); HRMS m/z calcd. for $C_{20}H_{31}ON_4$ [M + H]: 343.2492. Found 343.2483.

N-((1-(3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl)-1H-1,2,3-triazol-4-yl)methylene)-1-phenylmethanamine oxide (**6g**)

White solid, Purity: 96.8%; Yield: 74%, m.p.: 180–182 °C, IR (KBr, cm^{-1}): 2899 (HC=C), 1565 (−C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 2.79–2.81 (m, 2H, CH_2), 4.72 (t, J = 7.5 Hz, 2H, CH_2), 5.08 (s, 2H, CH_2N), 7.40–7.48 (m, 5H, Ar-H), 7.85 (s, 1H, Ar-H), 8.91 (s, 1H, HC=N); MS (ESI, 70 eV): 549 m/z (M + H); HRMS m/z calcd. for $C_{18}H_{13}ON_4F_{13}Na$ [M + Na]: 571.0774. Found 571.0755.

N-((1-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptadecafluorodecyl)-1H-1,2,3-triazol-4-yl)methylene)-1-phenylmethanamine oxide (**6h**)

White solid, Purity: 96.2%; Yield: 77%, m.p.: 194–196 °C, IR (KBr, cm^{-1}): 2992 (HC=C), 1588 (−C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 2.72–2.90 (m, 2H, CH_2), 4.70 (t, J = 6.7 Hz, 2H, CH_2), 5.08 (s, 2H, CH_2N), 7.41–7.49 (m, 5H, Ar-H), 7.85 (s, 1H, Ar-H), 8.91 (s, 1H, HC=N); MS (ESI, 70 eV): 649 m/z (M + H); HRMS m/z calcd. for $C_{20}H_{14}F_{17}N_4O$ [M + H]: 649.0890. Found 649.0896.

2-Methyl-N-((1-phenyl-1H-1,2,3-triazol-4-yl)methylene)propan-2-amine oxide (**6i**)

White solid, Purity: 98.8%; Yield: 89%, m.p.: 144–146 °C, IR (KBr, cm^{-1}): 3011 (HC=C), 1541 (−C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 1.65 (s, 9H, $3CH_3$), 7.45 (t, J = 7.1 Hz, 1H, Ar-H), 7.54 (t, J = 8.1 Hz, J = 7.1 Hz, 2H, Ar-H), 7.81 (d, J = 8.1 Hz, 2H, Ar-H), 8.12 (s, 1H, Ar-H), 9.35 (s, 1H, HC=N); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 140.9, 136.6, 129.7, 128.8, 123.4, 123, 120.3, 70.09, 28.03; MS (ESI, 70 eV): 245 m/z (M + H); HRMS m/z calcd. for $C_{13}H_{17}ON_4$ [M + H]: 245.1396. Found 245.1390.

N-((1-(4-Fluorophenyl)-1H-1,2,3-triazol-4-yl)methylene)-2-methylpropan-2-amine oxide (**6j**)

White solid, Purity: 98.2%; Yield: 85%, m.p.: 145–146 °C, IR (KBr, cm^{-1}): 2911 (HC=C), 1591 (−C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 1.64 (s, 9H, $3CH_3$), 7.22 (d, J = 9.06 Hz, 2H, Ar-H), 7.78 (d, J = 9.06 Hz, 2H, Ar-H), 8.13 (s, 1H, Ar-H), 9.31 (s, 1H, HC=N); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 163.6, 161.7, 141.3, 133.2, 123.3, 117.2, 116.9, 70.4, 28.03; MS (ESI, 70 eV): 285 m/z (M + Na); HRMS m/z calcd. for $C_{13}H_{15}FN_4ONa$ $[M + Na]^+$: 285.1122. Found 285.1116.

N-((1-(4-Methoxyphenyl)-1H-1,2,3-triazol-4-yl)methylene)-2-methylpropan-2-amine oxide (**6k**)

White solid, Purity: 98.9%; Yield: 84%, m.p.: 108–110 °C, IR (KBr, cm^{-1}): 2875 (HC=C), 1566 (−C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 1.64 (s, 9H, $3CH_3$), 3.87 (s, 3H, OCH_3), 7.04 (d, J = 9.1 Hz, 2H, Ar-H), 7.70 (d, J = 9.1 Hz, 2H, Ar-H), 8.12 (s, 1H, Ar-H), 9.25 (s, 1H, HC=N); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 159.8, 140.7, 130.0, 123.5, 123.2, 121.9, 114.8, 69.9, 55.5, 28.0; MS (ESI, 70 eV): 297 m/z (M + Na); HRMS m/z calcd. for $C_{14}H_{19}O_2N_4$ [M + H]: 275.1502. Found 275.1494.

2-Methyl-N-((1-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methylene)propan-2-amine oxide (**6l**)

White solid, Purity: 97.6%; Yield: 88%, m.p.: 156–158 °C, IR (KBr, cm^{-1}): 2911 (HC=C), 1591 (−C=N), 1287 (C–F); ^1H NMR (DMSO-d_6, 300 MHz): δ 1.65 (s, 9H, $3CH_3$), 7.82 (d, J = 9.06 Hz, 2H, Ar-H), 7.98 (d, J = 9.06 Hz, 2H, Ar-H), 8.15 (s, 1H, Ar-H), 9.44 (s, 1H, HC=N); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 141.6, 139.3, 130.1, 127.5, 123.3, 123.03, 120.5, 70.6, 28.3; MS (ESI, 70 eV): 313 m/z (M + H); HRMS m/z calcd. for $C_{14}H_{16}ON_4F_3$ [M + H]: 313.1270. Found 313.1263.

2-Methyl-N-((1-octyl-1H-1,2,3-triazol-4-yl)methylene)propan-2-amine oxide (**6m**)

White solid, Purity: 98.8%; Yield: 82%, m.p.: 76–78 °C, IR (KBr, cm^{-1}): 2968 (HC=C), 1596 (−C=N); ^1H NMR (DMSO-d_6,

300 MHz): δ 0.87 (t, J = 6.8 Hz, 3H, CH_3), 1.23–1.36 (m, 10H, –$(CH_2)_5$–), 1.62 (s, 9H, $3CH_3$), 1.88–1.96 (m, 2H, CH_2), 4.38 (t, J = 7.5 Hz, 2H, CH_2), 8.06 (s, 1H, Ar-H), 8.85 (s, 1H, HC=N); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 140.4, 125.5, 123.9, 69.9, 50.6, 31.9, 30.2, 28.9, 28.2, 26.6, 22.8, 14.2; MS (ESI, 70 eV): 281 *m/z* (M + H); HRMS *m/z* calcd. for $C_{15}H_{28}ON_4Na$ [M + Na]: 303.2155. Found 303.2145.

2-Methyl-N-((1-decyl-1H-1,2,3-triazol-4-yl)methylene)propan-2-amine oxide (**6n**)

White solid, Purity: 98.9%; Yield: 85%, m.p.: 82–84 °C, IR (KBr, cm^{-1}): 2956 (HC=C), 1549 (–C=N); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.88 (t, J = 6.7 Hz, 3H, CH_3), 1.22–1.34 (m, 14H, –$(CH_2)_7$–), 1.62 (s, 9H, $3CH_3$), 1.88–1.96 (m, 2H, CH_2), 4.38 (t, J = 7.5 Hz, 2H, CH_2), 8.05 (s, 1H, Ar-H), 8.85 (s, 1H, HC=N); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 140.2, 125.3, 123.8, 69.8, 50.4, 31.8, 30.0, 28.9, 28.0, 26.4, 22.6, 14.0; MS (ESI, 70 eV): 331 *m/z* (M + Na); HRMS *m/z* calcd. for $C_{17}H_{32}N_4ONa$ [M + Na]: 331.2468. Found 331.2461.

2-Methyl-N-((1-(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-1H-1,2,3-triazol-4-yl)methylene)propan-2-amine oxide (**6o**)

White solid, Purity: 97.4%; Yield: 71%, m.p.: 140–142 °C, IR (KBr, cm^{-1}): 2896 (HC=C), 1556 (–C=N), 1278 (C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.62 (s, 9H, $3CH_3$), 2.73–2.92 (m, 2H, CH_2), 4.71 (t, J = 7.5 Hz, 2H, CH_2), 8.06 (s, 1H, Ar-H), 8.96 (s, 1H, HC=N); MS (ESI, 70 eV): 537 *m/z* (M + Na); HRMS *m/z* calcd. for $C_{15}H_{16}ON_4F_3$ [M + H]: 515.1111. Found 515.1089.

N-((1-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptadecafluorodecyl)-1H-1,2,3-triazol-4-yl)methylene)-2-methylpropan-2-amine oxide (**6p**)

White solid, Purity: 97.2%; Yield: 74%, m.p.: 164–166 °C, IR (KBr, cm^{-1}): 2945 (HC=C), 1578 (–C=N), 1289 (C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.62 (s, 9H, $3CH_3$), 2.73–2.91 (m, 2H, CH_2), 4.70 (t, J = 7.3 Hz, 2H, CH_2), 8.06 (s, 1H, Ar-H), 8.94 (s, 1H, HC=N); MS (ESI, 70 eV): 615 *m/z* (M + H); HRMS *m/z* calcd. for $C_{17}H_{16}F_{17}N_4O$ ($[M + H]^+$): 615.1047. Found 615.1051.

4.8. THP-1 cell culture

THP-1 monocytes were obtained from ATCC and grown in RPMI 1640 (Sigma) containing 10% FBS (Lonza), non essential amino acids, penicillin (100 U/mL) and streptomycin (100 mg/mL) in 100 mM dishes (SPL life sciences), incubated at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air.

4.9. Measurement of cell viability

The effect of synthesized compounds on cell viability was determined by Trypan blue dye exclusion assay. THP-1 cells were seeded at a density of 2 × 10^5 cells/mL in 24-well plates in triplicates and were treated with 20 μM concentrations of **5a**–**e** and **6a**–**p** compounds in the absence of PMA. After 48 h time period, cells were harvested and re-suspended in 0.4% Trypan blue (Invitrogen) and live/dead cells were counted using cell counting chamber (Invitrogen).

4.10. Enzyme-linked immunosorbent assay for IL-1β inhibition

To check the inhibitory effects of compounds **5a**–**e** and **6a**–**p** on PMA-induced inflammation, THP-1 monocytes were seeded at a density of 2 × 10^5 cells/mL in 12 well plates. Cells were pre-treated with 5, 10 and 20 μM concentrations of compounds for 2 h before they were stimulated with 100 nM PMA. After 48 h, the supernatants were harvested and assayed for IL-1β levels using an enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's instructions (eBiosciences, San Diego, CA, USA) [31]. The absorbance in each well was measured on a microplate reader at 450 nm and corrected at 570 nm. IC50 values of test compounds on IL-1β production were calculated as described in previous paper [31].

4.11. In vitro cytotoxicity assay

4.11.1. Procedure

The cytotoxicity was measured using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] according to the method of Mossman [32]. Briefly, the cells (2 × 10^4) were seeded in each well containing 0.1 ml of medium in 96 well plates. After overnight incubation at 37 °C in 5% CO_2, the cells were treated with 100 μl of different test concentrations of test compounds (2–20 μg) at identical conditions with three replicates each. The final test concentrations were equivalent to 10–100 μM. The cell viability was assessed after 24 h, by adding 10 μl of MTT (5 mg/ml) per well. The plates were incubated at 37 °C for additional three hours. The medium was discarded and the formazan blue formed in the cells was dissolved with 100 μl of DMSO. The rate of color formation was measured at 570 nm using the TRIAD multimode reader (Dynex Technologies, Inc., Chantilly, VA). The IC_{50} values (50% inhibitory concentration) were calculated from the plotted absorbance data for the dose–response curves. IC_{50} values (in μM) are indicated as means ± SD of three independent experiments.

4.12. Caspase-3 assay

The caspase-3 activity of the active compound (**6h**) was measured using AFC conjugated Ac-DEVD as a substrate [33]. The human prostate cancer cells (PC-3) were seeded in 6 well plates with the cell confluence of 2.5 × 10^5 per well and incubated for 24 h. Cells were treated with the active compound (**6 h**) at two different concentrations, 8 μM (IC_{50} value) and 10 μM (>IC_{50} value), along with the standard Doxorubicin at 1 μM and 2 μM, respectively. After treatment, the cells were trypsinized and centrifuged at 3000 × *g* for 10 min at 4 °C. Cells were lysed with lysis buffer (HEPES 250 mM, CHAPS 25 mM, DTT 25 mM) followed by incubation on ice for 20–30 min. The lysate was centrifuged at 16,000 × *g* for 20 min at 4 °C and the obtained supernatant was used for measurement of caspase activity. In a 96 well black polystyrene plate, 2 μl of caspase-3 substrate and 1:1 ratio of supernatant and 2 × assay buffer (HEPES 200 mM, CHAPS 1%, EDTA 20 mM, DTT 50 mM) were loaded and the reaction was allowed to take place for 1 h. The fluorescence generated by the release of the fluorogenic group AFC on cleavage by caspase-3 was measured by excitation at 400 nm and emission at 505 nm wavelength for every 5 min over a period of 1 h. The liberated fluorescence was normalized to cell lysate protein using Bradford's protein estimation method.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.04.052.

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