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Solvent free synthesis of 1,5-disubstituted tetrazoles derived from Baylis Hillman acetates as potential TNF-α inhibitors

P. Srihari [a,*], Palash Dutta [a], R. Srinivasa Rao [a], J. S. Yadav [a], S. Chandrasekhar [a], P. Thombare [b], J. Mohapatra [b], A. Chatterjee [b], Mukul R. Jain [b,*]

[a] Organic Chemistry Division-I, Indian Institute of Chemical Technology, Hyderabad 500 607, India
[b] Zydus Research Centre, Sarkhej-Bavla N.H. No 8A, Moraiya, Ahmedabad 382 213, India

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ABSTRACT

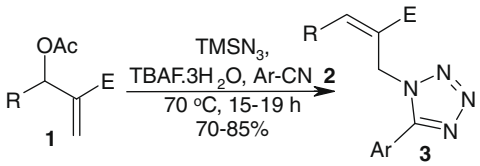
Solvent free multicomponent reaction of Baylis Hillman acetate, TMS azide and arylnitrile to produce 1,5-disubstituted tetrazole is described. Some of these tetrazoles are found to be potential TNF-α inhibitors.

Tumor necrosis factor α (TNFα) is a potent proinflammatory cytokine which by binding to its receptors, TNFR1 and TNFR2, results in recruitment of signal transducers that activates at least two transcription factors, AP-1 and NF-κB.[1] The transcription factors in turn regulate the production of many proinflammatory cytokines and related proteins that are elevated in immune-inflammatory diseases. As excessive or unregulated TNF-α production implicates either in exacerbating/mediating number of disease states, thus decreasing TNF-α levels may become a valuable therapeutic strategy for the treatment of many inflammatory, infectious, immunological, or malignant diseases such as rheumatoid arthritis and psoriasis.[2]

Recently click chemistry has been much focused wherein condensation of alkynes with azides occur in presence of copper to yield the triazoles.[3] However, similar addition of cyanides with azides to yield tetrazoles has not been much explored. Even though, tetrazole containing molecules are not available naturally and does not exhibit appreciable biological activity, they are found to resist biological degradation, and this property has enabled tetrazoles to form the isosteric substituent for various functional groups in the development of biologically active substances.[4] Tetrazoles have been also found to have wide applications in propellants,[5] explosives,[6] and pharmaceuticals.[7]

As part of our ongoing project for design and synthesis of small molecules as therapeutics in rheumatoid arthritis, we investigated the synthesis of novel tetrazoles as new chemical entities and their activities towards the inhibition of tumor necrosis factor α. We herein report the multicomponent addition reaction of Baylis Hillman acetates[8] with azide followed by one pot click reaction with nitriles to yield 1,5-disubstituted tetrazoles and evaluation towards inhibition of tumor necrosis factor.

Our initial studies began with the addition of NaN_3 to Baylis Hillman acetate (**1a, 1c**) followed by aryl nitriles in the presence of metal salts of CuCl or $ZnCl_2$.[9] We chose DMF as the solvent and found out that the reaction works with copper chloride to yield the corresponding tetrazoles in ~50% yield along with ~10–15% of the intermediate azido products. However, in the case of zinc chloride, there was no reaction and the starting material was completely recovered. We also investigated TBAF as the catalyst for this transformation as this reagent is well known for promoting



R = CH_3, Ph, *p*-$MeOC_6H_4$, MeC_6H_4, furyl
Ar= Ph, *m*-BrC_6H_4, 3-pyridyl, 4-pyridyl
E= CO_2Et, CN

Scheme 1.

* Corresponding authors. Tel.: +91 4027191490 (P.S.).
E-mail address: srihari@iict.res.in (P. Srihari).

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Table 1
In-vitro TNF-α inhibition at 100 μM and 10 μM concentration in human whole blood assay

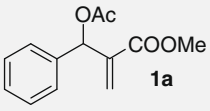
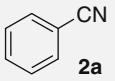
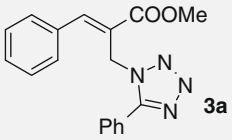
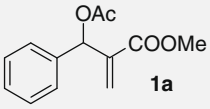
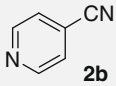
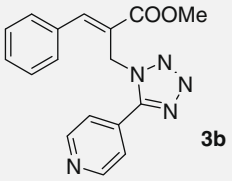
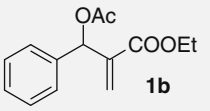
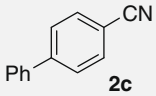
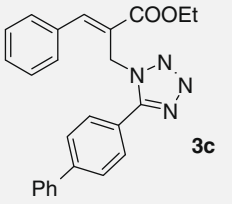
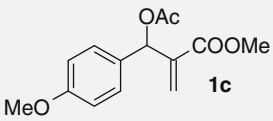
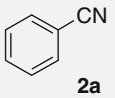
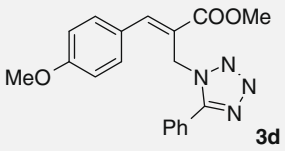
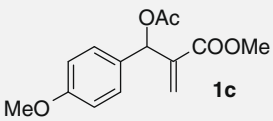
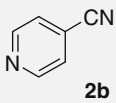
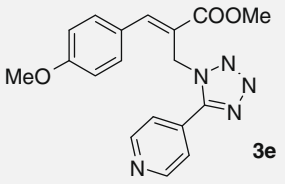
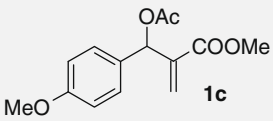
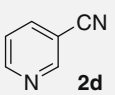
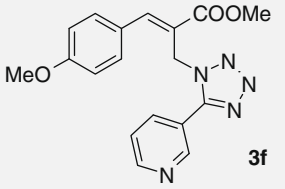
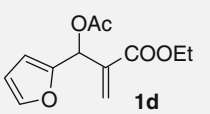
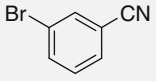
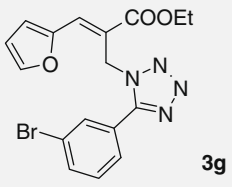
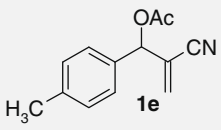
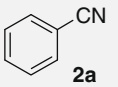
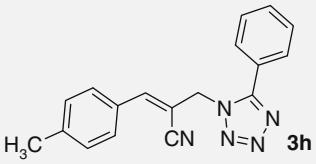
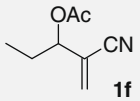
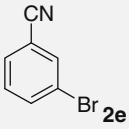
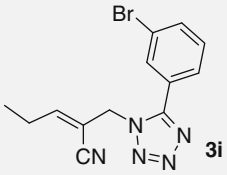
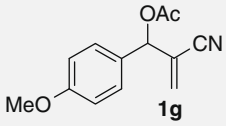
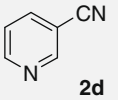
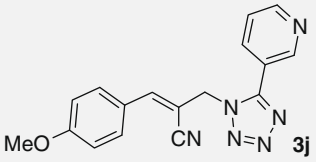
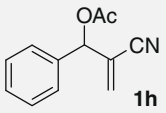
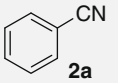
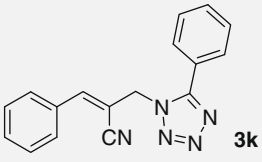
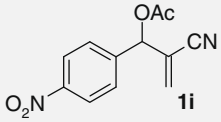
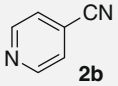
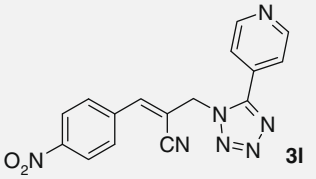
Entry	Baylis Hillmann acetates	Nitriles	Product[a]	Product ratio[b] (*E*:*Z*)	Time (h)	Yield[c] (%)
1	 **1a**	 **2a**	 **3a**	100:0	18	75
2	 **1a**	 **2b**	 **3b**	100:0	15	72
3	 **1b**	 **2c**	 **3c**	100:0	16	75
4	 **1c**	 **2a**	 **3d**	100:0	18	78
5	 **1c**	 **2b**	 **3e**	100:0	16	80
6	 **1c**	 **2d**	 **3f**	100:0	19	82
7	 **1d**	 **2e**	 **3g**	100:0	15	85

Table 1 (*continued*)

Entry	Baylis Hillmann acetates	Nitriles	Product[a]	Product ratio[b] (*E*:*Z*)	Time (h)	Yield[c] (%)
8	 **1e**	 **2a**	 **3h**	17:83	18	70
9	 **1f**	 **2e**	 **3i**	10:90	17	72
10	 **1g**	 **2d**	 **3j**	24:76	16	75
11	 **1h**	 **2a**	 **3k**	38:62	12	80
12	 **1i**	 **2b**	 **3l**	23:77	18	10

[a] All products were characterized by ^{1}H NMR, ^{13}C NMR, mass and IR spectroscopy.
[b] Product ratio determined by crude ^{1}H NMR spectra.
[c] Calculated after purification by column chromatography.

both Michael reactions[10] as well as click chemistry.[11] Thus we started with adduct **1a** (1 mmol), which was treated with $TMSN_3$ (1 mmol) in presence of TBAF in DMF and benzonitrile (1 mmol) at 85 °C for 18 h. A neat spot was observed which was isolated and characterized as the tetrazole **3a** in 65% yield. There was 5–10% increase in yield when the solvent was changed from DMF to THF or toluene.

Interestingly, the reaction also worked well in absence of solvent to give the product in 75% yield (Table 1 entry 1) and hence we chose to evaluate further scope of the reaction without any solvent media (Scheme 1). Thus, different Baylis Hillman acetates with ester moiety were investigated with several aryl nitriles (**2a**–**e**) in the presence of trimethylsilyl azide and found to produce the corresponding tetrazoles (**3a**–**g**) in good yields (Table 1).[12,13]

Even the Baylis Hillman acetates (BHA) with nitrile moiety (**1e**–**i**) reacted similarly to produce the corresponding substituted tetrazoles (**3h**–**l**). Substitution in aromatic ring of BHA did not change the yields significantly. However, the reaction of nitro containing aryl Baylis Hillman adduct **1i** gave poor yield when treated with 4-cyano pyridine **2b** (entry 12). It was found that *E* isomer was the only product formed with Baylis Hillman acetates containing ester moiety. Whereas nitrile containing Baylis Hillman acetates ended up with mixture of diastereomers with *Z* isomer as the major product.[14] The overall yield when compared for individual reactions for Michael reaction and Click reaction were found to be less than the one pot Michael followed by Click reaction.

Towards our goal for identification of small molecules for rheumatoid arthritis therapy, few of these new small molecules (substituted tetrazoles) synthesized (**3a**, **3b**, **3d**–**f**) were tested for in vitro TNF-α-inhibition studies[15] (Table 2) and found that compound **3b** and **3d** were significantly active.

In conclusion, we have developed a new strategy for synthesis of functionalized 1,5-disubstituted tetrazoles starting from Baylis Hillman acetates under solvent free reaction conditions. Based on

Table 2
In-vitro TNF-α inhibition at 100 μM and 10 μM concentration in human whole blood assay

S.No.	Compound	% of TNF-α inhibition (SEM)	
		100 μM	10 μM
1	**3a**	35.1 (1.9)	14.6 (1.8)
2	**3b**	67.0 (3.6)	18.2 (12.1)
3	**3d**	46.3 (5.6)	22.2 (0.4)
4	**3e**	12.9 (24.0)	8.6 (7.0)
5	**3f**	30.2 (3.0)	5.4 (19.8)

the preliminary reports obtained by us, further evaluation for SAR/lead identification towards arthritis is currently being investigated.

Acknowledgements

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12. *General procedure for multicomponent reaction*: Baylis Hillman acetate (1 mmol), $TBAF{\cdot}3H_2O$ (0.5 mmol), and $TMSN_3$ (1 mmol) were stirred at room temperature for half an hour and to this mixture was added aryl nitrile (1 mmol) and the mixture was heated to 85 °C till completion of the reaction (as given in Table 1). The compound was diluted with ethyl acetate and water was added. The aqueous layer was extracted with ethyl acetate. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography.
13. *Analytical data for compounds* **3a**: light green sticky liquid; IR (neat): *ν* 2925, 2850, 1711, 1604, 1512, 1459, 1256, 1178, 838, 754 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): *δ* 8.2 (s, 1H), 8.14–8.19 (m, 2H), 7.54–7.59 (m, 2H), 7.50–7.40 (m, 6H), 5.64 (s, 2H), 3.81 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 166.4, 164.9, 146.3, 133.7, 130.2, 129.8, 129.1, 128.9, 128.7, 126.8, 123.9, 113.8, 52.5, 49.9; MS-ESIMS: *m/z* 321 ($M+H^+$); HRMS calcd for $C_{18}H_{16}N_4O_2$: 343.1183, found 343.1170. Compound **3b**: Dark brown sticky liquid; IR (neat): *ν* 2921, 2851, 1715, 1611, 1456, 1257, 1206, 1106, 834, 757 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): *δ* 8.67 (s, 2H), 8.11 (s, 1H), 7.93 (s, 2H), 7.30–7.50 (m, 5H), 5.58 (s, 2H), 3.71 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 166.1, 162.7, 150.2, 146.4, 134.6, 133.3, 129.8, 128.8, 123.4, 120.7, 52.3, 50.1; MS-ESIMS: *m/z* 344 ($M+Na^+$); HRMS calcd for $C_{17}H_{15}N_5O_2$: 344.1133, found 344.1123. Compound **3c**: light green sticky liquid; IR (neat): *ν* 2928, 1711, 1219, 772 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): *δ* 8.23 (d, *J* = 8.3 Hz, 2H), 8.17 (s, 1H), 7.69 (d, *J* = 8.3 Hz, 2H), 7.64–755 (m, 4H), 7.48–7.39 (m, 5H), 7.38–7.31 (m, 1H), 5.64 (s, 2H), 4.27 (q, *J* = 6.7 Hz, 2H), 1.30 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 165.9, 164.7, 146.0, 142.9, 140.2, 133.8, 129.7, 129.0, 128.9, 128.8, 127.7, 127.4, 127.2, 127.0, 126.2, 124.3, 61.5, 49.9, 14.1; MS-ESIMS: *m/z* 411 ($M+H^+$); HRMS calcd for $C_{25}H_{23}N_4O_2$: 411.1815, found 411.1816. Compound **3d**: light green sticky liquid; IR (neat): *ν* 2922, 2848, 1709, 1601, 1511, 1442, 1254, 1176, 1026, 836, 731 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): *δ* 8.19–8.14 (m, 2H), 8.13 (s, 1H), 7.55 (d, *J* = 8.6 Hz, 2H), 7.47 (m, 3H), 6.95 (d, *J* = 8.8 Hz, 2H), 5.68 (s, 2H), 3.83 (s, 3H), 3.81 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 166.8, 164.9, 161.0, 146.1, 131.3, 130.2, 128.7, 127.3, 126.8, 126.1, 121.5, 114.4, 55.3, 52.4, 50.1; MS-ESIMS: *m/z* 373 ($M+Na^+$); HRMS calcd for $C_{19}H_{18}N_4O_3$: 373.1276, found 373.1271. Compound **3e**: light green sticky liquid; IR (neat): *ν* 2921, 2851, 1714, 1634, 1442, 1252, 1026, 805, 731 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): *δ* 8.77 (d, *J* = 4.3 Hz, 2H), 8.16 (s, 1H), 8.04 (d, *J* = 6.0 Hz, 2H), 7.54 (d, *J* = 8.6 Hz, 2H), 6.96 (d, *J* = 8.8 Hz, 2H), 5.72 (s, 2H), 3.84 (s, 3H), 3.82 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 166.7, 162.9, 161.1, 150.4, 146.4, 134.7, 131.2, 125.9, 121.1, 120.8, 114.4, 55.3, 52.4, 50.4; MS-ESIMS: *m/z* 352 ($M+H^+$); HRMS calcd for $C_{18}H_{17}N_5O_3$: 352.1411, found 352.1404. Compound **3f**: white crystalline solid, mp: 105–108 °C; IR (KBr): *ν* 2929, 1538, 1401, 1350, 1220, 1182, 840, 772 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): *δ* 9.30 (s, 1H), 8.63 (s, 1H), 8.35 (dt, *J* = 7.9, 1.7 Hz, 1H), 8.06 (s, 1H), 7.46 (d, *J* = 8.6 Hz, 2H), 7.30–7.38 (m, 1H), 6.88 (d, *J* = 8.6 Hz, 2H), 5.63 (s, 2H), 3.75 (s, 3H), 3.73 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 166.7, 162.6, 161.0, 151.0, 148.0, 146.2, 134.0, 131.2, 125.9, 123.5, 121.1, 114.4, 55.3, 52.4, 50.2; MS-ESIMS: *m/z* 374 ($M+Na^+$); HRMS calcd for $C_{18}H_{17}N_5O_3$: 374.1239, found 374.1229. Compound **3g**: white crystalline solid, mp: 105–108 °C; IR (KBr): *ν* 2924, 2854, 1705, 1636, 1446, 1252, 1212, 1098, 1021, 885, 764 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): *δ* 8.25 (t, *J* = 2.2 Hz, 1H), 8.05 (dt, *J* = 8.3, 1.5 Hz, 1H), 7.74 (s, 1H), 7.60–7.50 (m, 2H), 7.31 (t, *J* = 7.5 Hz, 1H), 6.84 (d, *J* = 8.3 Hz, 1H), 6.53 (dd, *J* = 3.7, 2.2 Hz, 1H), 5.96 (s, 2H), 4.26 (q, *J* = 7.5 Hz, 2H), 1.30 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 166.1, 163.3, 149.8, 146.1, 132.8, 130.5, 129.5, 129.3, 125.2, 122.6, 119.2, 118.5, 112.4, 61.3, 49.7, 14.0; MS-ESIMS: *m/z* 403 ($M+H^+$); HRMS calcd for $C_{17}H_{15}N_4O_3Br$: 403.0409, found 403.0327. Compound **3h**: white crystalline solid, mp: 93–95 °C; IR (KBr): *ν* 2924, 2853, 2211, 1604, 1446, 1343, 1187, 1022, 926, 811, 727, 698 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): *δ* 8.19–8.14 (m, 2H), 7.72 (d, *J* = 8.1 Hz, 2H), 7.53–7.45 (m, 3H), 7.31 (s, 1H), 7.25 (d, *J* = 7.5 Hz, 2H), 5.52 (s, 2H), 2.39 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 165.8, 149.1, 142.5, 130.5, 129.7, 129.4, 129.2, 128.8, 126.9, 116.7, 101.7, 56.1, 29.6, 21.5; MS-ESIMS: *m/z* 324 ($M+Na^+$); HRMS calcd for $C_{18}H_{15}N_5$: 324.1236, found 324.1225. Compound **3i**: pale yellow sticky liquid; IR (neat): *ν* 2924, 2853, 2210, 1458, 1219, 1038, 771.cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): *δ* 8.28 (d, *J* = 1.5 Hz, 1H), 8.08 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.59 (d, *J* = 8.3 Hz, 1H), 7.35 (t, *J* = 7.5 Hz, 1H), 6.58 (t, *J* = 7.5 Hz, 1H), 5.34 (s, 2H), 2.58–2.43 (m, 2H), 1.16 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 164.4, 156.4, 133.4, 130.4, 129.8, 128.7, 125.4, 122.9, 114.7, 107.4, 54.5, 25.2, 12.5; MS-ESIMS: *m/z* 318 ($M+H^+$); HRMS calcd for $C_{12}H_{12}BrN_5$: 318.0348, found 318.0335. Compound **3j**: white crystalline solid, mp: 122–125 °C; IR (KBr): *ν* 2924, 2853, 2201, 1741, 1599, 1513, 1434, 1262, 1180, 1025, 829, 750 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): *δ* 9.39 (d, *J* = 1.5 Hz, 1H), 8.71 (dd, *J* = 5.2, 1.5 Hz, 1H), 8.46 (dt, *J* = 7.5, 1.5 Hz, 1H), 7.57 (s, 1H), 7.52 (d, *J* = 9.0 Hz, 2H), 7.46–7.40 (m, 1H), 6.99 (d, *J* = 9.0 Hz, 2H), 5.61 (s, 2H), 3.87 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 163.4, 161.7, 151.4, 150.0, 148.1, 134.2, 131.3, 124.6, 123.6, 118.2, 114.7, 104.2, 55.4, 51.0, 29.6; MS-ESIMS: *m/z* 341 ($M+Na^+$); HRMS calcd for $C_{17}H_{14}N_6O$: 341.1139, found 341.1126. Compound **3k**: amorphous yellow solid, mp: 99–103 °C; IR (KBr): *ν* 2924, 2854, 2215, 2096, 1740, 1447, 1337, 1272, 1036, 849, 775, 688 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): *δ* 8.19–8.14 (m, 2H), 7.81 (dd, *J* = 6.0, 2.2 Hz, 2H), 7.52–7.41 (m, 6H), 7.25 (s, 1H), 5.51 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 165.8, 149.0, 131.9, 131.7, 130.5, 129.4, 129.0, 128.8, 126.9, 116.4, 103.2, 56.0, 29.6; MS-ESIMS: *m/z* 288 ($M+H^+$); HRMS calcd for $C_{17}H_{13}N_5$: 288.1248, found 288.1244. Compound **3l**: pale yellow sticky liquid; IR (KBr): *ν* 2923, 2853, 2212, 1740, 1604, 1522, 1461, 1346, 1261, 1099, 799, 652 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): *δ* 8.78 (dd, *J* = 4.5, 1.5 Hz, 2H), 8.34 (d, *J* = 8.3 Hz, 2H), 8.06–7.91 (m, 4H), 7.31 (s, 1H), 5.59 (s, 2H); MS-ESIMS: *m/z* 334 ($M+H^+$); HRMS calcd for $C_{16}H_{11}N_7O_2$: 334.1052, found 334.1047.
14. The diastereomeric mixture of a representative sample (**3j**) was separated and their respective geometries were characterized by NOE studies.
15. *Procedure for assay*: inhibition of TNF-α in human whole blood assay: fresh blood was collected aseptically in the presence of heparin by venipuncture from healthy adult volunteers. Two microliters of either a test compound solution (10, 100 μM) or dimethyl sulfoxide was mixed with 246-μl aliquot of blood and incubated at 37 °C for 1 h. Following this, 2 μl of 125 ng/ml lipopolysaccharide (dissolved in phosphate-buffered saline; final concentration of 1 ng/ml) were added in each microtube. The blood mixture along with LPS was further incubated at 37 °C for 5 h. The reactions were terminated by placing the samples over ice for 10 min. At study completion, the plasma was separated by centrifugation at 3000 rpm for 10 min at 4 °C and stored at −70 °C until further analysis. Concentrations of tumor necrosis factor-alpha in the plasma were determined by enzyme-linked immunosorbent assays (BD Biosciences, USA).