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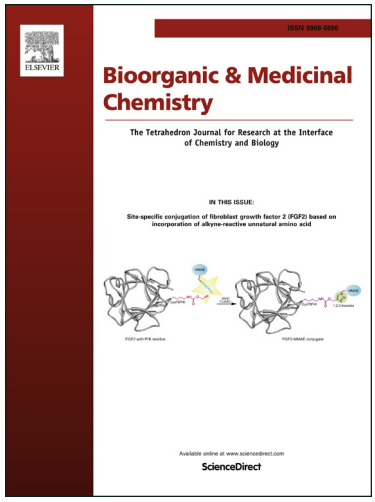

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Facile Alkylation of 4-Nitrobenzotriazole and its Platelet Aggregation Inhibitory Activity

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Abstract: We explored the facile alkylation of 4-nitrobenzotriazole under basic conditions and the synthesized derivatives were tested for their potential ADP induced platelet aggregation inhibition activity in comparison with standard drug ticagrelor (selective P2Y12 inhibitor). The nitro group at 4-position is highly activating towards alkylation reactions (under strong basic conditions) and resulted in formation of degradation product like 3-nitrobenzene-1,2-diamine which make isolation of alkyl products very difficult. We optimized the reaction under mild basic condition (potassium carbonate and DMF) which is devoid of any degradation product. This is perhaps the first report of 4-nitrobenzotriazole derivatives possessing platelet aggregation inhibitory activity. Generally activity increases with increase in length of alkyl chain and 1-alkyl positional isomers were found to be more potent than 2-alkyl isomers. The benzoyl derivative was found to be the most potent [compound 22; (4-Nitro-1*H*-benzotriazol-1-yl)(phenyl)methanone; IC_{50} = 0.65 ± 0.10 mM] which may be attributed to electronegative oxygen atom and aromatic ring. Benzyl derivatives [compound 20; 1-Benzyl-4-nitro-1*H*-benzotriazole; IC_{50} = 0.81 ± 0.08 mM, compound 21; 2-Benzyl-4-nitro-2*H*-benzotriazole; IC_{50} = 0.82 ± 0.19 mM) and sulfonyl derivative [compound 23; 1-[(4-Methylphenyl)sulfonyl]-4-nitro-1*H*-benzotriazole; IC_{50} = 0.82 ± 0.19 mM] are also found to be highly active. Furthermore, all compounds possess P2Y12 binding affinity as confirmed by VASP/P2Y12 phosphorylation assay.

Keywords: Alkylation of nitrobenzotriazole; 4-nitrobenzotriazole; 3-Nitrobenzene-1,2-diamine; Platelets; Ticagrelor

1. Introduction

The benzotriazole is well explored motif to have versatile medicinal effects [1]. Recently, its sodium hydrogen exchanger inhibitory activity is reported [2]. Moreover, benzotriazoles are imperative in inter-disciplinary science fields as they possess corrosion inhibitory property [3, 4], optical properties [5], lubricating [6], and synthetic phyto-hormonal activity [7]. Katritzky *et al* well explored the chemistry of benzotriazole moiety for many decades [8-10], although 4-nitro benzotriazoles is relatively less explored [11, 12].

Platelets play imperative role in hemostasis as they stop bleeding from damaged blood vessels. However, abnormal hemostasis can cause platelet aggregation which in turn could lead to the formation of blood clots. Hence, there is unmet need to develop platelet aggregation inhibitors [13, 14]. Adenosine-5'-diphosphate (ADP) is an important mediator of platelet activation and aggregation [15]. ADP acts on two receptors of platelets: P2Y1 involved in platelet shape change and activation while P2Y12 involved in aggregation of platelets [16]. P2Y12R belongs to P2Y purinergic GPCR family stimulated by ADP and this receptor plays crucial role in platelet activation, aggregation, and thrombus formation [17, 18]. Inhibition of ADP induced platelet aggregation is an indicator of selective inhibition of ADP binding to P2Y12 [19].

There are number of platelet inhibitory drugs like clopidogrel, prasugrel, but they have very long half-life and are prodrugs which contribute to their side effects. Then, nucleoside analogs came into existence like cangrelor, but because of phosphate groups, it has very short half life and thus, they require intravenous administration. Hence, structural modifications lead to triazolopyrimidine analogs like ticagrelor with advantage of its metabolic stability and increase binding affinity [20, 21]. The Platelet Inhibition and Patient Outcomes (PLATO) trials showed that ticagrelor had antithrombotic effects that were superior to those of clopidogrel in patients with acute coronary syndromes [22].

Benzotriazole structure is similar to imidazopyrimidine of ADP as well as ticagrelor's triazolopyrimidine, therefore we modified benzotriazole ring by introducing nitro group at 4^{th} position and subsequent addition of alkyl groups at 1^{st} and 2^{nd} position. Herein, we prepared series of nitro benzotriazole derivatives and tested for its potential platelet aggregation inhibitory activity. Moreover, all synthesized compounds were evaluated for their P2Y12 binding affinity.

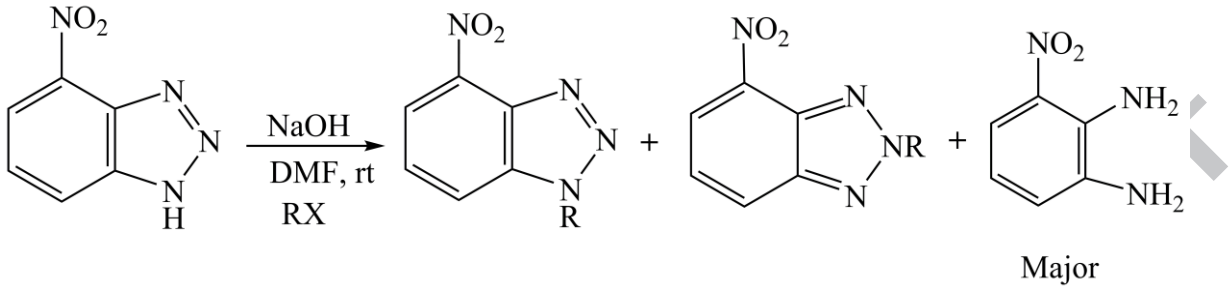
Alkylation of benzotriazole is a well reported reaction involving nucleophilic substitution of benzotriazole nitrogen on alkyl halides or dimethyl sulphate under basic conditions [10, 23]. Alkylation of 4-nitrobenzotriazole is relatively less explored in comparison to alkylation of 5-nitrobenzotriazole [24]. Diehl *et al* reported the alkylations of 4-nitrobenztriazole, but most derivatives synthesized were cyclic saturated, few N-alkyls like isopropyl derivatives. Indeed, the reported reactions completed in 30 hours [25]. Few alkyl reactions may be attributed to the fact that alkylation of 4-nitrobenzotriazole result in impurities formation and tedious separation of isomers. We primarily explored facile alkylation of 4-nitrobenzotriazole in potassium carbonate and DMF in only 30 minutes with absence of impurity. Hence, with incessant growing research in this field, there is imperative need to explore this reaction.

2. Results and Discussion

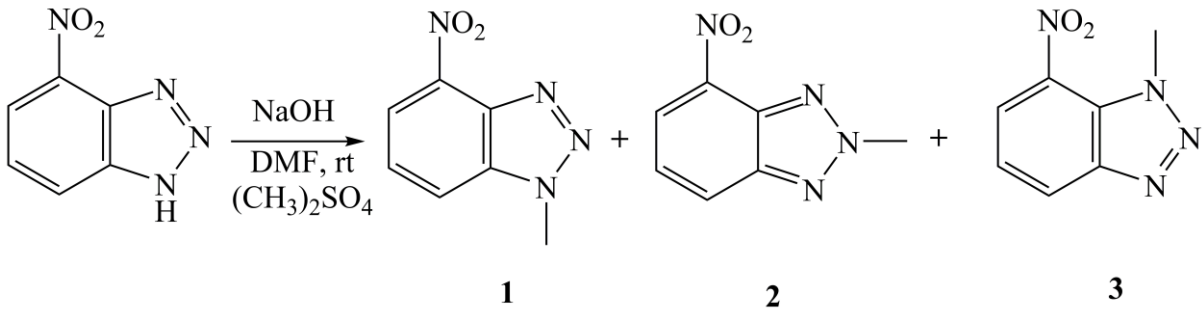
2.1. Chemistry

Alkylation of 4-nitrobenzotriazole (10 mmol) was carried out using sodium hydroxide (40 mmol) and alkyl halides (10 mmol) in N,N-Dimethylformamide (DMF) (8-10 mL) solution as shown in scheme-1 [26]. Three isomers of 4-nitrobenzotriazole methyl derivative were reported in DMF solvent and described in Scheme-2 [10].

During this reaction a yellow colored compound was always formed with the desired products (alkyl isomers) formed by all different alkyl halides. Indeed, in thin layer chromatography the spot of this yellow compound was spread over the spots of isomers and present in between the isomers. Thus, it hindered with the isolation of isomer products, although isomers and impurity (yellow compound) could be isolated by using silica gel of mesh size 400-700. But it is very tedious procedure. As this impurity was common in all alkyl halides, it was suspected to be degradation product of 4-nitrobenzotriazole. So, a reaction of 4-nitrobenzotriazole with sodium hydroxide and DMF-without addition of alkyl halides, was carried out at room temperature, but did not result in yellow compound formation. Further on heating this solution above 45^{o}C resulted in degradation of 4-nitrobenzotriazole and same yellow compound was isolated. Thus, reaction of alkyl halides with 4-nitrobenzotriazole in sodium hydroxide and DMF was tried under cold conditions, but yellow impurity was still found, besides the isomer products. Upon NMR analysis impurity was found to be 3-Nitrobenzene-1, 2-diamine.

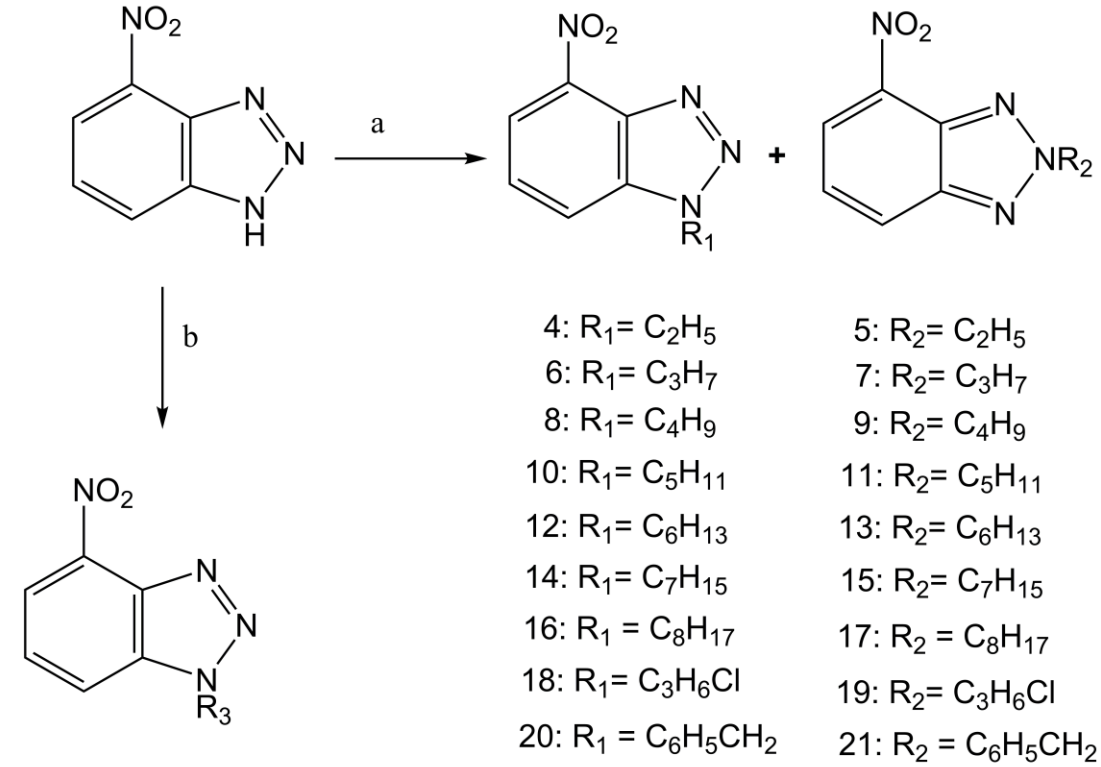


Scheme-1: Alkylation under strong basic conditions.



Scheme-2: Methylation under strong basic conditions.

Consequently, alkylation was carried out under milder conditions using potassium carbonate and dry DMF for 30 minutes stirring at room temperature. Here, alkyl products from methyl to octyl, chloropropyl, benzyl, benzoyl, and tosyl derivatives were prepared and isolated as described in Scheme-3.



Scheme-3: Alkylation under mild basic conditions. Reagents and Conditions: (a) Potassium Carbonate, DMF, Stirred for 30 minutes, RX: C_2H_5Br (4, 5), C_3H_7Br (6, 7), C_4H_9Br (8, 9), $C_5H_{11}Br$ (10, 11), $C_6H_{13}Br$ (12, 13), $C_7H_{15}Br$ (14, 15), C_8H_{17} (16, 17), $BrCH_2CH_2$ CH_2Cl (18, 19), $C_6H_5CH_2Cl$ (20, 21); (b) Pyridine, Reflux, 30 min., ArCl: C_6H_5COCl (22), $C_7H_7SO_2Cl$ (23).

2.2. Docking

We used iGEMDOCK for docking studies using 4-NTJ (P2Y12R). The result indicated that all compounds bind to the same pocket as ticagrelor as indicated in **Figure 1**. Ligand map and interaction residues of ticagrelor are indicated in **Figure 2**. Moreover, they bind to same critical residues that are observed as hot spots, and are indicated in **Table 1** [21]. All the compounds fit into same binding pocket and their interactions energies are summarized in **Table 2**. For each interacting residue, Z score is a measure of interaction conservation between the interacting groups and screening compounds. Z score and pharmacological preference, and Pharmacological interaction profile tables are provided in supplementary information.

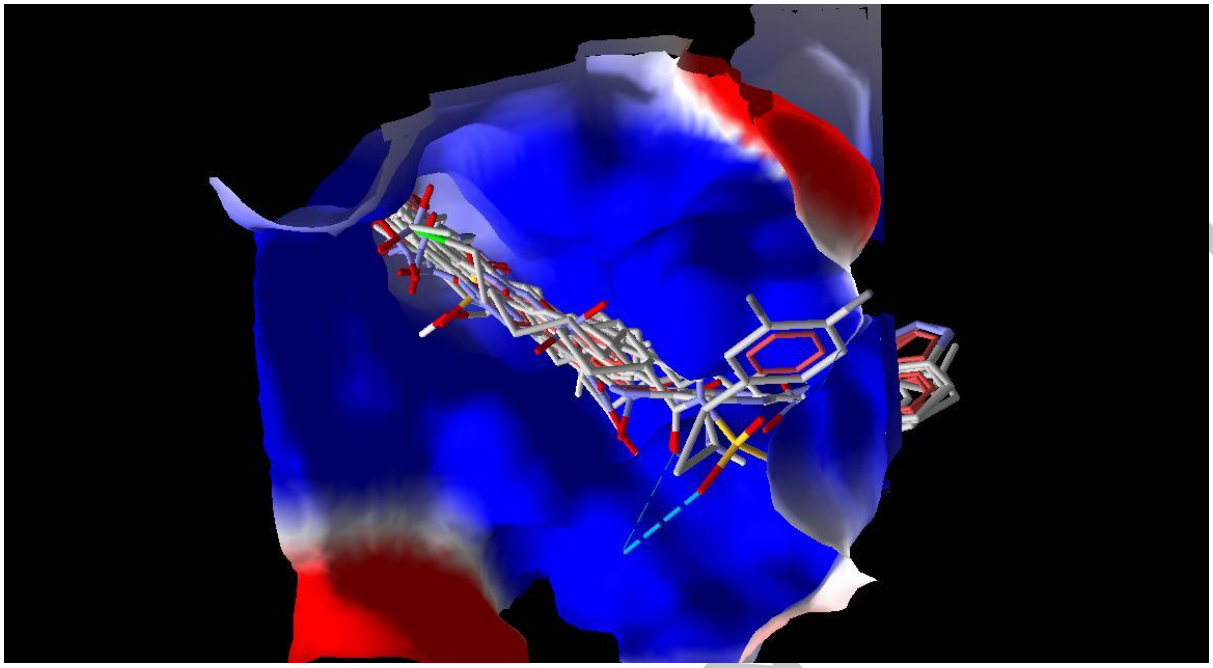


Figure 1: Figure showing all compounds bind to same pocket as standard drug ticagrelor.

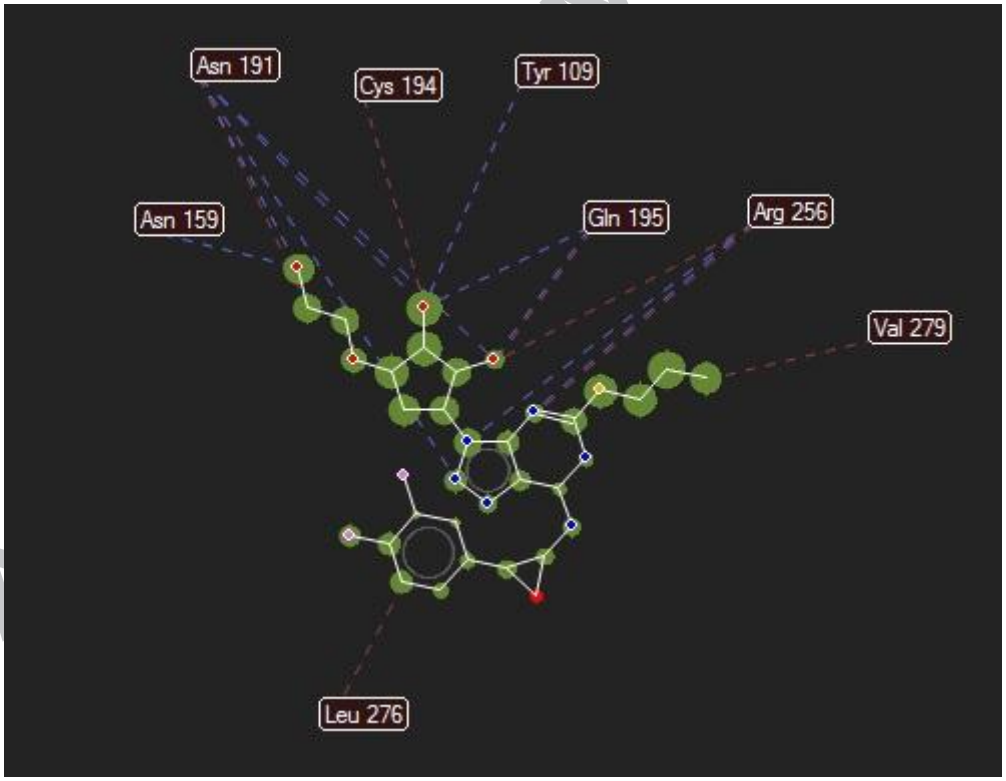


Figure 2: Figure showing interactions residues and ligand map of ticagrelor. Blue, green, and red lines indicates hydrogen bonding, electrostatic, and steric interactions respectively with residue.

Table 1: Table showing Pharmacological interactions, W score, consensus interaction, and Hot spot (indicating residues critical for interaction).

Predicted Pharmacological Interaction	Pharmacological Preference	Consensus Ratio	Hot Spot
H-S SER156	0.86	0.50	Yes
H-S ASN191	0.34	0.34	No
H-S CYS194	1.00	0.53	Yes
V-M VAL102	0.38	0.65	Yes
V-M TYR105	0.70	0.73	Yes
V-S TYR105	1.00	0.92	Yes
V-S TYR109	0.96	0.88	Yes
V-S VAL190	0.57	0.65	Yes
V-S ASN191	0.71	0.77	Yes

Table 2: Total energy in Kcal/Mol. Van der Waals interactions (VDW, Kcal/Mol), Hydrogen bonding (HBond, Kcal/Mol), electrostatic interactions (Elec, Kcal/Mol), average conpair, and rank (Based on total energy). Total energy = VDW + HBond + Elec

#Ligand	TotalEnergy	VDW	HBond	Elec	AverConPair	Rank
4-NO_2BTZ	-81.052	-66.684	-14.665	0.298	35.333	25
BTZ	-75.177	-60.423	-14.754	0	39.778	26
Ticagrelor	-135.259	-113.664	-21.596	0	21.722	1
Compound 1	-82.2944	-65.2992	-16.9952	0	33.3077	24
Compound 2	-82.339	-65.326	-17.014	0	34.615	23
Compound 3	-84.049	-71.864	-12.185	0	34.846	22
Compound 4	-90.597	-63.855	-27.505	0.763	33.929	19
Compound 5	-89.592	-72.630	-16.963	0	35.000	21
Compound 6	-91.688	-75.215	-16.473	0	32.800	18
Compound 7	-94.941	-74.844	-20.097	0	34.867	15
Compound 8	-95.946	-80.232	-15.714	0	31.812	14

Compound 9	-93.353	-80.118	-13.235	0	33.188	17
Compound 10	-99.0164	-84.231	-14.7855	0	31.3529	10
Compound 11	-96.288	-81.660	-15.095	0.467	31.824	13
Compound 12	-97.896	-77.472	-21.138	0.714	29.444	12
Compound 13	-98.576	-80.946	-17.900	0.270	28.000	11
Compound 14	-102.074	-86.944	-15.997	0.866	29.684	9
Compound 15	-103.023	-96.881	-6.449	0.306	31.737	8
Compound 16	-105.139	-88.751	-17.434	1.0463	27.600	6
Compound 17	-103.548	-88.953	-15.098	0.504	27.100	7
Compound 18	-93.540	-80.941	-12.599	0	33.062	16
Compound 19	-90.359	-77.413	-12.945	0	32.312	20
Compound 20	-113.147	-99.847	-13.300	0	33.210	3
Compound 21	-107.183	-94.309	-13.358	0.484	31.684	4
Compound 22	-115.186	-99.705	-15.481	0	32.100	2
Compound 23	-105.663	-94.990	-10.673	0	30.773	5

2.3. Biological Activity

The platelet aggregation inhibitory activity of all synthesized compounds was determined using ADP-induced platelet aggregation test *in-vitro* [27, 28]. Inhibition of ADP-induced platelet aggregation is directly correlated with inhibition of P2Y12 receptor present on platelets. The results are expressed as the half-maximal inhibitory concentration (IC_{50}) values presented in **Table 3**; the mean values of experiments were performed in triplicate. The benzotriazole possesses platelet aggregation inhibitory activity and its derivatives could mimic the imidazopyrimidine of ADP as well as triazolopyrimidine of ticagrelor (selective P2Y12 inhibitor). The introduction of nitro group increased the activity by more than three times. The activity increases with increase in alkyl chain length octyl derivatives (1-Octyl-4-nitro-1*H*-benzotriazole; IC_{50} = 0.91 ± 0.43 mM) is more potent than heptyl (1-Heptyl-4-nitro-1*H*-benzotriazole; IC_{50} = 0.93 ± 0.92 mM) ones and 1-isomers were found to be more potent

than 2-isomers except methyl derivative. Introduction of aromatic ring result in marked increase in potency like benzoyl [(4-Nitro-1*H*-benzotriazol-1-yl)(phenyl)methanone; IC_{50} = 0.65 ± 0.10 mM], benzyl (1-Benzyl-4-nitro-1*H*-benzotriazole; IC_{50} = 0.81 ± 0.08 mM, 2-Benzyl-4-nitro-2*H*-benzotriazole; IC_{50} = 0.82 ± 0.19 mM) and tosyl derivatives (1-[(4-Methylphenyl)sulfonyl]-4-nitro-1*H*-benzotriazole; IC_{50} = 0.86 ± 0.14 mM). The introduction of polar chloro group in alkyl chain i.e., 3-chloropropyl (1-(3-Cholropropyl)-4-nitro-1*H*-benzotriazole; IC_{50} = 1.07 ± 0.96 mM) was found to be more active than propyl analogue (1-Propyl-4-nitro-1*H*-benzotriazole; IC_{50} = 1.30 ± 0.35 mM).

Table 3: Platelet aggregation inhibitory activity of compounds (**1-23**)

Compounds	IC_{50}[a,b]
Ticagrelor	0.32 ± 0.02[c]
Benzotriazole	7.43 ± 0.23
4-Nitrobenzotriazole	2.06 ± 0.20
Compound 1	1.84 ± 0.36
Compound 2	1.96 ± 0.27
Compound 3	1.80 ± 0.49
Compound 4	1.50 ± 0.32
Compound 5	1.62 ± 0.54
Compound 6	1.30 ± 0.35
Compound 7	1.32 ± 0.97
Compound 8	1.05 ± 0.97
Compound 9	1.07 ± 0.87
Compound 10	1.07 ± 0.96
Compound 11	1.02 ± 0.81
Compound 12	1.04 ± 0.93
Compound 13	0.94 ± 1.29
Compound 14	0.95 ± 1.33
Compound 15	0.93 ± 0.92
Compound 16	0.94 ± 1.06
Compound 17	0.92 ± 0.81
Compound 18	0.91 ± 0.43

Compound 19	1.26 ± 0.17
Compound 20	0.81 ± 0.08
Compound 21	0.82 ± 0.19
Compound 22	0.65 ± 0.10
Compound 23	0.86 ± 0.14

[a] IC_{50} is reported in mM (required to produce 50% inhibition in ADP induced platelet aggregation); [b] n=3; [c] IC_{50} is reported in µM

2.3.1. P2Y12 Binding Assay:

P2Y12 binding assay was carried out using CY-QUANT VASP/P2Y12 ELISA kit. This test is highly selective and equivalent to flow cytometric PLT VASP/P2Y12 assay [29-33]. All compounds were tested at 200 µM concentration and inhibited the effect of ADP on PGE1 induced phosphorylation. The PGE1 induced phosphorylation was set as 100% and ADP inhibited this phosphorylation completely. All compounds were able to block effect of ADP on this PGE1 induced phosphorylation (**Table 4**), indicating their specific binding (antagonist) to P2Y12. Maximum effect was observed for the benzoyl derivative, followed by benzyl derivatives, indicating the role of aromatic ring in the binding. No *per se* effect at 200 µM concentration was observed for all compounds. Hence, indicating that compounds do not possess P2Y12 agonistic activity.

Table 4: Percent Phosphorylation of compounds (**1-23**)

Compounds	% Phosphorylation[a]
Ticagrelor	98.2 ± 7.5
Benzotriazole	8.1 ± 23.6
4-Nitrobenzotriazole	19.3 ± 22.4
Compound 1	20.4 ± 8.2
Compound 2	20.8 ± 9.8
Compound 3	21.8 ± 8.7
Compound 4	22.5 ± 9.3
Compound 5	21.6 ± 10.5
Compound 6	26.9 ± 12.4
Compound 7	27.1 ± 11.8
Compound 8	34.3 ± 10.5
Compound 9	33.8 ± 10.7

Compound 10	33.7 ± 10.6
Compound 11	32.7 ± 15.9
Compound 12	33.2 ± 13.6
Compound 13	36.1 ± 17.4
Compound 14	37.4 ± 13.1
Compound 15	40.2 ± 9.7
Compound 16	37.4 ± 18.0
Compound 17	39.4 ± 12.1
Compound 18	37.8 ± 13.4
Compound 19	27.4 ± 11.7
Compound 20	40.3 ± 10.7
Compound 21	41.8 ± 8.5
Compound 22	43.8 ± 9.3
Compound 23	39.4 ± 11.8

[a] n=3

3. Conclusions

Consequently, alkylation of 4-nitrobenzotriazole in sodium hydroxide was tedious, perhaps due to severe conditions in sodium hydroxide result in degradation and it precedes alkylation. Hence, the reactions were carried out in milder basic conditions; potassium carbonate in DMF. Alkylation of 5-nitrobenzotriazole with potassium carbonate and DMF with stirring for overnight to 24 hours is reported recently, while Diehl *et al* reported reaction of 4-nitrobenzotriazole with sodium hydroxide and acetonitrile in 30 hours. In 4-nitrobenzotriazole, the reaction is completed within 30 minutes indicating regioactivation toward nucleophilic substitution of benzotriazole by 4-nitro group which is more activating than 5-nitro group. The ADP-induced platelet aggregation inhibitory activity of benzotriazole derivatives may be due to structural similarity with the imidazopyrimidine of ADP as well as ticagrelor triazolopyrimidine. The introduction of nitro group increased the activity by more than three times. Hence, bolstering the hypothesis that the derivatives of 4-nitrobenzotriazole possess platelet aggregation inhibitory activity. The activity increases with increase in alkyl chain length: octyl derivatives (Compound 18; IC_{50} = 0.91 ± 0.43 mM) was more potent than methyl (Compound 2; IC_{50} = 1.96 ± 0.27 mM). Amongst the positional isomers, 1-isomers were found to be more potent than 2-isomers except for the methyl derivative. The

introduction of polar chloro group in alkyl chain i.e., 3-chloropropyl (Compound 10; IC_{50} = 1.07 ± 0.96 mM) was found to be more active than propyl analogue (Compound 6; IC_{50} = 1.30 ± 0.35 mM). Introduction of aromatic ring result in marked increase in potency like benzoyl (Compound 22; IC_{50} = 0.65 ± 0.10 mM), benzyl (Compound 20; IC_{50} = 0.81 ± 0.08 mM) and tosyl derivatives (Compound 21; IC_{50} = 0.82 ± 0.19 mM) and thus indicating the role of aromatic ring in the P2Y12 binding. These three compounds could be further modified in order to achieve equipotent compound as ticagrelor.

4. Experimental Section

4.1. Chemistry general

Melting points reported are uncorrected. Synthetic procedures employed were monitored for completion of product formation by Thin Layer Chromatography (TLC) employing 7 cm x 2.5 cm Silica gel 60 F_{254} precoated TLC plates by Merck. Nuclear Magnetic Resonance Spectra were recorded on Bruker Avance DPX-200 (400MHz). In ^{1}H-NMR chemical shifts (δ) are reported in parts per million (ppm) using tetramethylsilane as internal standard. Coupling constants (J) are reported in Hz (Hertz). Multiplicities are reported using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, sx = sextet, qu = quintet. Waters Micromass Q-tof Micromass Spectrometer for HRMS. Waters HPLC system equipped with a pump, an autosampler, a Dual UV detector set at 300 nm, and an analytical column (Waters, XTerra, RP18, 4.6 mm × 50 mm, 5 µm). The mobile phase consisted of varying ratios of HPLC grade acetonitrile and triple distilled water The flow rate was set at 1 mL/min. IR spectra were measured on a Hitachi 270-30 infrared and Bruker Vector 22 Spectrophotometers. IR spectra were recorded as KBr pellets.

4.1.1. General Procedure for Synthesis 4-nitrobenzotriazole. To cool solution of 2.65 mL of nitric acid and 10 mL sulfuric acid was added drop wise in a solution of 5.37 g benzotriazole in 10 mL sulfuric acid. The reaction was kept at room temperature overnight. The reaction mixture was then over ice and yellow precipitates were formed which are filtered, washed with water, and dried. The purification was done by slurrying of nitrobenzotriazole in hot acetonitrile and product was filtered [25].

4.1.2. General Procedure for Synthesis of Alkyl derivatives of 4-nitrobenzotriazole (**1-21**)**.** To the mixture of 4-nitrobenzotriazole 10 mmol and 40 mmol dry potassium carbonate, 8-10 mL dry N,N-dimethylformamide was added. Corresponding 10 mmoles of alkyl halides were added and stirred for 30 minutes [26]. The mixture was washed with water. 1- and 2-

alkyl substituted derivatives were separated using column chromatography (Silica gel #200-400, Petroleum ether: Ethyl acetate, 95:05).

4.1.3. General Procedure for Synthesis of Acyl/Sulfonyl derivatives (22, 23). To the mixture of 1.00 mmol of 4-nitrobenzotriazole in 3 mL dry pyridine, 1.80 mmoles of benzoyl chloride, tosyl chloride were added in small proportions and resultant mixture was heated to boiling for 30 minutes. After completion of the reaction, the reaction mixture was acidified with 2M HCl and poured on crushed ice [34]. Precipitated product was filtered and washed well with water. The product was recrystallized using ethanol (95 %) to give compounds 22 and 23. The acylation of benzotriazole forms thermodynamically more stable 1-acyl derivative [35].

4.1.4. 4-Nitro-1H-benzotriazole. Yellow Solid; yield: 5.37 g (73%); m.pt. 228-230 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 16.62 (broad s, 1H, ArH), 8.57-8.55 (d, J = 8.16 Hz, 1H, Ar H), 8.45-8.43 (d, J = 7.8 Hz, 1H, Ar H), 7.66-7.62 (t, J = 8.04 Hz, 1H, Ar H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 140.02, 139.63, 125.13, 123.66, 118.65, 108.95; HRMS: 165.1214 [ES+]; Exact mass calcd for $C_6H_4N_4O_2$ 164.1215 [m/z]; IR (cm^{-1}) 3418, 3247, 1512, 1479, 1337, 1178, 1024, 926, 851, 684, 542; HPLC Purity 100 % at 300 nm, t_R= 2.837 min.

*4.1.5. 1-Methyl-4-nitro-1H-benzotriazole (**1**).* 58 mg; Creamish Solid; m.pt. 203 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.31-8.29 (d, J = 7.64 Hz, 1H, ArH), 7.94-7.92 (d, J = 8.4 Hz, 1H, Ar H), 7.70-7.66 (t, J = 8.0 Hz, 1H, Ar H), 4.43 (s, 3H, $\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 138.48, 135.84, 126.66, 121.29, 116.26, 34.94; HRMS: 179.1477 [ES+]; Exact mass calcd for $C_7H_6N_4O_2$ 178.1481 [m/z]; IR (cm^{-1}) 3082, 1965, 1855, 1522, 1451, 1399, 1346, 1288, 1059, 885, 822, 736, 577; HPLC Purity 99.61 % at 300 nm, t_R= 2.931 min.

*4.1.6. 2-Methyl-4-nitro-2H-benzotriazole (**2**).* 32 mg; Creamish Solid; m.pt. 184 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.43-8.41 (d, J = 8.16 Hz, 1H, ArH), 8.37-8.35 (d, J = 7.88 Hz, 1H, Ar H), 7.53-7.49 (t, J = 8.04 Hz, 1H, Ar H), 4.61 (s, 3H, $\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 149.16, 138.43, 126.17, 122.13, 118.59, 39.46; HRMS: 179.1479 [ES+]; Exact mass calcd for $C_7H_6N_4O_2$ 178.1481 [m/z]; IR (cm^{-1}) 3068, 1960, 1837, 1519, 1457, 1392, 1338, 1281, 1055, 883, 819, 731, 568; HPLC Purity 100 % at 300 nm, t_R= 2.945 min.

*4.1.7. 1-Methyl-7-nitro-1H-benzotriazole (**3**).* 10 mg; Brownish Solid; m.pt. 113 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.43-8.41 (d, J = 7.56 Hz, 1H, ArH), 8.28-8.26 (d, J = 8.84 Hz, 1H, Ar H), 7.57-7.53 (t, J = 8.16 Hz, 1H, Ar H), 4.66 (s, 3H, $\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ

141.16, 125.02, 124.67, 120.70, 108.41, 31.78; HRMS: 179.1480 [ES+]; Exact mass calcd for $C_7H_6N_4O_2$ 178.1481 [m/z]; IR (cm^{-1}) 3064, 1943, 1851, 1504, 1453, 1397, 1348, 1286, 1054, 885, 814, 734, 572; HPLC Purity 98.51 % at 300 nm, t_R= 2.844 min.

*4.1.8. 1-Ethyl-4-nitro-1H-benzotriazole (**4**).* 57 mg; Yellow-Creamish Solid; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.42-8.40 (d, *J* = 7.68 Hz, 1H, ArH), 8.29-8.26 (d, *J* = 8.46 Hz, 1H, Ar H), 7.56-7.52 (t, *J* = 8.08 Hz, 1H, Ar H), 4.96-4.91 (q, *J* = 7.36 Hz, 2H, N-$\underline{CH_2}$), 1.80-1.76 (t, *J* = 7.4 Hz, 3H, N-$\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 142.36, 127.74, 126.46, 121.20, 119.15, 64.83, 29.66, 21.76; HRMS: 193.1746 [ES+]; Exact mass calcd for $C_8H_8N_4O_2$ 192.1747 [m/z]; IR (cm^{-1}) 3078, 1961, 1852, 1517, 1446, 1395, 1341, 1281, 1054, 881, 827, 733, 572; HPLC Purity 98.45 % at 300 nm, t_R= 2.18 min.

*4.1.9. 2-Ethyl-4-nitro-2H-benzotriazole (**5**).* 33 mg; Yellow-Creamish Solid; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.44-8.42 (d, *J* = 8.24 Hz, 1H, ArH), 8.37-8.35 (d, *J* = 7.84 Hz, 1H, Ar H), 7.53-7.49 (t, *J* = 8.04 Hz, 1H, Ar H), 5.11-5.06 (q, *J* = 7.2 Hz, 2H, N-$\underline{CH_2}$), 1.57-1.54 (t, *J* = 7.2 Hz, 3H, N-$\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 147.28, 125.81, 124.29, 118.94, 113.62, 65.02, 21.13, 18.41; HRMS: 193.1743 [ES+]; Exact mass calcd for $C_8H_8N_4O_2$ 192.1747 [m/z]; IR (cm^{-1}) 3085, 1963, 1852, 1527, 1446, 1395, 1342, 1289, 1054, 887, 829, 732, 574; HPLC Purity 99.39 % at 300 nm, t_R= 2.03 min.

*4.1.10. 1-Propyl-4-nitro-1H-benzotriazole (**6**).* 69 mg; Yellow Oil; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.42-8.40 (d, *J* = 7.62 Hz, 1H, ArH), 8.29-8.26 (d, *J* = 8.46 Hz, 1H, Ar H), 7.56-7.52 (t, *J* = 8.08 Hz, 1H, Ar H), 4.86-4.82 (t, *J* = 7.24 Hz, 2H, N-$\underline{CH_2}$), 2.24-2.19 (sx, *J* = 7.32 Hz, 2H, N-$\underline{CH_2}$), 1.04-1.00 (t, *J* = 7.49 Hz, 3H, N-$\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 143.46, 131.16, 129.04, 124.72, 114.19, 61.20, 27.96, 18.63; HRMS: 207.2009 [ES+]; Exact mass calcd for $C_9H_{10}N_4O_2$ 206.2013 [m/z]; IR (cm^{-1}) 3086, 2969, 2878, 1727, 1627, 1528, 1446, 1390, 1346, 1307, 1200, 1152, 1104, 1051, 1022, 987, 882, 821, 738, 595; HPLC Purity 100 % at 300 nm, t_R= 2.941 min.

*4.1.11. 2-Propyl-4-nitro-2H-benzotriazole (**7**).* 38 mg; Brownish Oil; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.30-8.28 (d, *J* = 7.68 Hz, 1H, ArH), 7.92-7.90 (d, *J* = 8.24 Hz, 1H, Ar H), 7.66-7.62 (t, *J* = 5.24 Hz, 1H, Ar H), 4.73-4.69 (t, *J* = 7.12 Hz, 2H, N-$\underline{CH_2}$), 2.12-2.06 (sx, *J* = 7.28 Hz, 2H, N-$\underline{CH_2}$), 1.02-0.98 (t, *J* = 7.44 Hz, 3H, N-$\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 144.72, 130.42, 127.84, 125.67, 115.36, 63.18, 28.71, 17.29; HRMS: 207.2011 [ES+]; Exact mass calcd for $C_9H_{10}N_4O_2$ 206.2013 [m/z]; IR (cm^{-1}) 2997, 2875, 1724, 1629, 1527, 1446, 1361, 1148, 1053, 984, 829, 735, 597; HPLC Purity 100 % at 300 nm, t_R= 2.861 min.

*4.1.12. 1-Butyl-4-nitro-1H-benzotriazole (**8**).* 72 mg; Light-Brown Oil; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.29-8.27 (d, *J* = 7.68 Hz, 1H, ArH), 7.93-7.91 (d, *J* = 8.16 Hz, 1H, Ar H), 7.67-7.63 (t, *J* = 7.96 Hz, 1H, Ar H), 4.76-4.73 (t, *J* = 7.12 Hz, 2H, N-$\underline{CH_2}$), 2.06-2.03 (qu, *J* = 7.36 Hz, 2H, N-$\underline{CH_2}$), 1.44-1.38 (t, *J* = 7.52 Hz, 2H, N-$\underline{CH_2}$), 1.00-0.96 (t, *J* = 7.32 Hz, 3H, N-$\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 146.45, 137.22, 127.66, 124.74, 123.03, 57.29, 52.21, 33.07, 19.80, 13.52; HRMS: 221.2277 [ES+]; Exact mass calcd for $C_{10}H_{12}N_4O_2$ 220.2278 [m/z]; IR (cm^{-1}) 3014, 2877, 1721, 1624, 1529, 1438, 1346, 1126, 1023, 973, 831, 727, 592; HPLC Purity 100 % at 300 nm, t_R= 3.064 min.

*4.1.13. 2-Butyl-4-nitro-2H-benzotriazole (**9**).* 39 mg; Dark-Brown Oil; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.41-8.39 (d, *J* = 7.64 Hz, 1H, ArH), 8.27-8.25 (d, *J* = 8.44 Hz, 1H, Ar H), 7.55-7.50 (t, *J* = 8.2 Hz, 1H, Ar H), 4.89-4.85 (t, *J* = 7.28 Hz, 2H, N-$\underline{CH_2}$), 2.20-2.16 (qu, *J* = 7.36 Hz, 2H, N-$\underline{CH_2}$), 1.45-1.38 (sx, *J* = 7.52 Hz, 2H, N-$\underline{CH_2}$), 1.01-0.97 (t, *J* = 7.36 Hz, 3H, N-$\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 145.34, 136.49, 126.13, 125.39, 124.74, 123.03, 52.21, 32.07, 19.65, 13.482; HRMS: 221.2278 [ES+]; Exact mass calcd for $C_{10}H_{12}N_4O_2$ 220.2278 [m/z]; IR (cm^{-1}) 3012, 2879, 1723, 1627, 1524, 1435, 1349, 1125, 1026, 976, 834, 724, 596; HPLC Purity 100 % at 300 nm, t_R= 2.885 min.

*4.1.14. 1-Pentyl-4-nitro-1H-benzotriazole (**10**).* 79 mg; Brownish Oil; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.44-8.42 (d, *J* = 8.96 Hz, 1H, ArH), 8.29-8.26 (d, *J* = 8.44 Hz, 1H, Ar H), 7.57-7.52 (t, *J* = 8.12 Hz, 1H, Ar H), 4.88-4.85 (t, *J* = 7.32 Hz, 2H, N-$\underline{CH_2}$), 2.22-2.14 (qu, *J* = 7.52 Hz, 2H, N-$\underline{CH_2}$), 1.42-1.33 (m, 4H, N-$\underline{CH_2CH_2}$), 0.93-0.89 (t, *J* = 6.88 Hz, 3H, N-$\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 146.44, 127.52, 126.16, 125.40, 124.38, 123.04, 57.56, 30.81, 28.63, 23.48, 13.85; HRMS: 235.2543 [ES+]; Exact mass calcd for $C_{11}H_{14}N_4O_2$ 234.2544 [m/z]; IR (cm^{-1}) 3027, 2972, 1734, 1624, 1537, 1432, 1352, 1129, 1028, 967, 837, 723, 596; HPLC Purity 99.89 % at 300 nm, t_R= 2.714 min.

*4.1.15. 2-Pentyl-4-nitro-2H-benzotriazole (**11**).* 37 mg; Dark-Brown Oil; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.41-8.39 (d, *J* = 7.68 Hz, 1H, ArH), 8.28-8.26 (d, *J* = 8.48 Hz, 1H, Ar H), 7.55-7.51 (t, *J* = 7.96 Hz, 1H, Ar H), 4.88-4.84 (t, *J* = 7.36 Hz, 2H, N-$\underline{CH_2}$), 2.22-2.14 (qu, *J* = 7.48 Hz, 2H, N-$\underline{CH_2}$), 1.45-1.30 (m, 4H, N-$\underline{CH_2CH_2}$), 0.93-0.89 (t, *J* = 6.76 Hz, 3H, N-$\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 145.84, 127.03, 125.61, 124.75, 124.01, 122.97, 52.46, 29.94, 28.52, 18.12, 12.91; HRMS: 235.2541 [ES+]; Exact mass calcd for $C_{11}H_{14}N_4O_2$ 234.2544 [m/z]; IR (cm^{-1}) 3024, 2975, 1724, 1622, 1526, 1437, 1348, 1124, 1026, 964, 835, 726, 598; HPLC Purity 96.74 % at 300 nm, t_R= 2.769 min.

*4.1.16. 1-Hexyl-4-nitro-1H-benzotriazole (**12**).* 83 mg; Brownish Oil; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.30-8.28 (d, *J* = 7.72 Hz, 1H, ArH), 7.92-7.89 (d, *J* = 8.24 Hz, 1H, Ar H), 7.66-7.62 (t, *J* = 8.0 Hz, 1H, Ar H), 4.75-4.71 (t, *J* = 7.16 Hz, 2H, N-$\underline{CH_2}$), 2.05-1.85 (qu, *J* = 7.16 Hz, 2H, N-$\underline{CH_2}$), 1.42-1.33 (m, 6H, N-$\underline{CH_2CH_2CH_2}$), 0.87-0.82 (t, *J* = 7.12 Hz, 3H, N-$\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 146.52, 128.83, 126.40, 121.14, 116.26, 108.39, 67.65, 48.96, 31.11, 29.76, 26.32, 13.91; HRMS: 249.2805 [ES+]; Exact mass calcd for $C_{12}H_{16}N_4O_2$ 248.2810 [m/z]; IR (cm^{-1}) 2952, 2927, 2868, 1724, 1528, 1459, 1347, 1307, 815, 740; HPLC Purity 97.43 % at 300 nm, t_R= 2.954 min.

*4.1.17. 2-Hexyl-4-nitro-2H-benzotriazole (**13**).* 22 mg; Yellowish Oil; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.42-8.39 (d, *J* = 7.7 Hz, 1H, ArH), 8.28-8.26 (d, *J* = 8.44 Hz, 1H, Ar H), 7.56-7.52 (t, *J* = 8.06 Hz, 1H, Ar H), 4.88-4.84 (t, *J* = 7.36 Hz, 2H, N-$\underline{CH_2}$), 2.21-2.14 (qu, *J* = 7.28 Hz, 2H, N-$\underline{CH_2}$), 1.69-1.32 (m, 6H, N-$\underline{CH_2CH_2CH_2}$), 1.31-1.29 (t, *J* = 3.36 Hz, 3H, N-$\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 145.59, 127.68, 125.13, 122.65, 116.19, 109.14, 66.93, 47.18, 30.49, 28.81, 26.73, 14.63; HRMS: 249.2808 [ES+]; Exact mass calcd for $C_{12}H_{16}N_4O_2$ 248.2810 [m/z]; IR (cm^{-1}) 2955, 2924, 2865, 1526, 1463, 1346, 1302, 813, 746; HPLC Purity 99.71 % at 300 nm, t_R= 2.974 min.

*4.1.18. 1-Heptyl-4-nitro-1H-benzotriazole (**14**).* 87 mg; Dark-Brown Oil; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.23-8.21 (d, *J* = 7.6 Hz, 1H, ArH), 7.86-7.83 (d, *J* = 8.38 Hz, 1H, Ar H), 7.60-7.56 (t, *J* = 7.88 Hz, 1H, Ar H), 4.99-4.83 (t, *J*= 7.16 Hz, 2H, N-$\underline{CH_2}$), 1.54-1.17 (m, 10H, N-$\underline{CH_2CH_2CH_2CH_2CH_2}$), 0.81-0.75 (t, *J* = 6.8 Hz, 3H, N-$\underline{CH_3}$); ^{13}C-NMR (100MHz, $CDCl_3$): δ 146.44, 137.21, 126.17, 125.40, 124.40, 123.04, 57.58, 52.49, 31.58, 31.11, 28.67, 26.36, 14.02; HRMS: 263.3075 [ES+]; Exact mass calcd for $C_{13}H_{18}N_4O_2$ 262.3076 [m/z]; IR (cm^{-1}) 3029, 2854, 1713, 1643, 1538, 1431, 1349, 1134, 1025, 964, 837, 714, 583; HPLC Purity 99.79 % at 300 nm, t_R= 3.188 min.

*4.1.19. 2-Heptyl-4-nitro-2H-benzotriazole (**15**).* 33 mg; Dark-Brown Oil; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.40-8.38 (d, *J* = 7.74 Hz, 1H, ArH), 8.27-8.25 (d, *J* = 8.48 Hz, 1H, Ar H), 7.55-7.51 (t, *J* = 8.1 Hz, 1H, Ar H), 4.68-4.65 (t, *J* = 7.4 Hz, 2H, N-$\underline{CH_2}$), 1.40-1.22 (m, 10H, N-$\underline{CH_2CH_2CH_2CH_2CH_2}$), 0.87-0.80 (t, *J* = 7.4 Hz, 3H, N-$\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 146.21, 136.80, 127.53, 125.03, 124.76, 122.16, 56.06, 51.70, 31.54, 30.15, 26.49, 22.52, 13.15; HRMS: 264.3073 [ES+]; Exact mass calcd for $C_{13}H_{18}N_4O_2$ 262.3076 [m/z]; IR (cm^{-1}) 3024, 2857, 1715, 1641, 1535, 1432, 1346, 1138, 1027, 961, 835, 718, 586; HPLC Purity 99.53 % at 300 nm, t_R= 2.937 min.

*4.1.20. 1-Octyl-4-nitro-1H-benzotriazole (**16**).* 93 mg; Dark-Brown Oil; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.29-8.27 (d, *J* = 7.44 Hz, 1H, ArH), 7.93-7.91 (d, *J* = 8.16 Hz, 1H, Ar H), 7.67-7.63 (t, *J* = 8.04 Hz, 1H, Ar H), 4.75-4.72 (t, *J* = 7.2 Hz, 2H, N-$\underline{CH_2}$), 2.05-1.21 (m, 12H, N-$\underline{CH_2CH_2CH_2CH_2CH_2CH_2}$), 0.87-0.83 (t, *J* = 6.68 Hz, 3H, N-$\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 141.34, 136.94, 128.02, 126.16, 124.75, 113.52, 57.58, 31.69, 30.14, 29.02, 28.95, 26.52, 22.59, 14.06; HRMS: 277.3339 [ES+]; Exact mass calcd for $C_{14}H_{20}N_4O_2$ 276.3342 [m/z]; IR (cm^{-1}) 3042, 2914, 1723, 1637, 1532, 1427, 1342, 1139, 1022, 963, 834, 723, 597; HPLC Purity 99.76 % at 300 nm, t_R= 3.276 min.

*4.1.21. 2-Octyl-4-nitro-2H-benzotriazole (**17**).* 24 mg; Dark-Brown Oil; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.41-8.39 (d, *J* = 7.64 Hz, 1H, ArH), 8.28-8.25 (d, *J* = 8.36 Hz, 1H, Ar H), 7.55-7.51 (t, *J* = 8.2 Hz, 1H, Ar H), 4.87-4.84 (t, *J* = 7.36 Hz, 2H, N-$\underline{CH_2}$), 2.19-1.25 (m, 12H, N-$\underline{CH_2CH_2CH_2CH_2CH_2CH_2}$), 0.88-0.83 (t, *J* = 6.64 Hz, 3H, N-$\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 142.85, 135.36, 126.42, 121.17, 116.36, 113.52, 48.97, 31.66, 29.79, 29.01, 28.93, 26.66, 22.57, 14.05; HRMS: 277.3337 [ES+]; Exact mass calcd for $C_{14}H_{20}N_4O_2$ 276.3342 [m/z]; IR (cm^{-1}) 3039, 2917, 1728, 1634, 1537, 1423, 1092, 965, 902, 692, 596; HPLC Purity 99.43 % at 300 nm, t_R= 3.059 min.

*4.1.22. 1-(3-Cholropropyl)-4-nitro-1H-benzotriazole (**18**).* 53 mg; Light-Brownish Oil; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.31-8.29 (d, *J* = 7.16 Hz, 1H, ArH), 8.02-8.00 (d, *J* = 7.84 Hz, 1H, Ar H), 7.71-7.67 (t, *J* = 8.02 Hz, 1H, Ar H), 4.95-4.92 (t, *J* = 6.56 Hz, 2H, N-$\underline{CH_2}$), 3.56-3.53 (t, *J* = 5.8 Hz, 2H, N-$\underline{CH_2}$Cl), 2.60-2.54 (qu, *J* = 6.48 Hz, 2H, N-$\underline{CH_2}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 146.47, 137.39, 127.79, 125.77, 124.66, 123.33, 54.42, 41.24, 33.55; HRMS: 241.6458 [ES+]; Exact mass calcd for $C_9H_9ClN_4O_2$ 240.6463 [m/z]; IR (cm^{-1}) 3064, 2863, 1928, 1782, 1657, 1583, 1397, 1095, 962, 812, 768, 747, 672, 583; HPLC Purity 98.51 % at 300 nm, t_R= 2.844 min.

*4.1.23. 2-(3-Cholropropyl)-4-nitro-2H-benzotriazole (**19**).* 47 mg; Dark-Brownish Oil; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.42-8.40 (d, *J* = 7.88 Hz, 1H, ArH), 8.29-8.27 (d, *J* = 8.58 Hz, 1H, Ar H), 7.58-7.54 (t, *J* = 8.04 Hz, 1H, Ar H), 5.08-5.05 (t, *J* = 6.76 Hz, 2H, N-$\underline{CH_2}$), 3.67-3.64 (t, *J* = 6.16 Hz, 2H, N-$\underline{CH_2}$Cl), 2.69-2.62 (qu, *J* = 6.56 Hz, 2H, N-$\underline{CH_2}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 145.26, 128.78, 126.88, 121.39, 116.22, 106.32, 45.32, 41.16, 32.27; HRMS: 240.6459 [ES+]; Exact mass calcd for $C_9H_9ClN_4O_2$ 240.6463 [m/z]; IR (cm^{-1}) 3063, 2879, 1925, 1786, 1652, 1584, 1394, 1092, 965, 817, 763, 741, 676, 587; HPLC Purity 97.09 % at 300 nm, t_R= 2.686 min.

*4.1.24. 1-Benzyl-4-nitro-1H-benzotriazole (**20**).* 52 mg; Creamish Solid; m.pt. 123-125 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.26-8.24 (d, *J* = 7.7 Hz, 1H, ArH), 7.71-7..69 (d, *J* = 8.4 Hz, 1H, Ar H), 7.56-7.52 (t, *J* = 7.84 Hz, 1H, Ar H), 7.37-7.34 (m, 3H-Benzyl), 7.29-7.26 (m, 2H-Benzyl), 5.96 (s, 2H, $\underline{CH_2}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 146.13, 129.81, 129.03, 128.46, 127.93, 127.02, 126.79, 125.61, 124.35, 123.60, 117.72, 58.16; HRMS: 255.2439 [ES+]; Exact mass calcd for $C_{14}H_{20}N_4O_2$ 254.2441 [m/z]; IR (cm^{-1}) 3084, 2974, 1897, 1532, 1476, 1327, 1126, 1084, 963, 902, 683, 592; HPLC Purity 99.90 % at 300 nm, t_R= 2.723 min.

*4.1.25. 2-Benzyl-4-nitro-2H-benzotriazole (**21**).* 28 mg; Creamish Solid; m.pt. 93-95 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.44-8.42 (d, *J* = 8.24 Hz, 1H, ArH), 8.23-8.21 (d, *J* = 7.76 Hz, 1H, Ar H), 7.54-7.48 (m, 1H-Ar, 1H-Benzyl), 7.38-7.37 (m, 2H-Benzyl), 7.26-7.24 (distorted t, *J* = 5.88 Hz, 1H-Benzyl), 7.09-7.07 (distorted t, *J* = 2.6 Hz, 1H-Benzyl), 6.01 (s, 2H, $\underline{CH_2}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 146.34, 129.94, 129.36, 128.2, 127.98, 127.11, 126.19, 125.43, 124.02, 123.14, 117.09, 57.42; HRMS: 255.2438 [ES+]; Exact mass calcd for $C_{14}H_{20}N_4O_2$ 254.2441 [m/z]; IR (cm^{-1}) 3083, 2978, 1895, 1536, 1475, 1329, 1123, 1081, 967, 905, 686, 594; HPLC Purity 99.04 % at 300 nm, t_R= 2.729 min.

*4.1.26. (4-Nitro-1H-benzotriazol-1-yl)(phenyl)methanone (**22**).* Yellowish Solid; yield: 84%; ^{1}H-NMR (400MHz, $CDCl_3$): δ 8.43-8.41 (d, *J* = 8.36 Hz, 1H, ArH), 8.25-8.23 (d, *J* = 8.56 Hz, 1H, Ar H), 7.53-7.48 (m, 2H, Ar H), 7.38-7.37 (d, *J* = 3.32 Hz, 1H-Ar), 7.26-7.22 (m, 2H-Ar), 7.08-7.06 (distorted t, *J* = 5.24 Hz, 1H-Ar); ^{13}C-NMR (100MHz, $CDCl_3$): δ 154.36, 147.82, 142.19, 134.61, 131.04, 129.57, 128.66, 127.16, 124.31, 122.96, 121.25, 116.49, 114.26; HRMS: 269.2268 [ES+]; Exact mass calcd for $C_{13}H_{18}N_4O_3$ 268.2272 [m/z]; IR (cm^{-1}) 3273, 1527, 1481, 1349, 1127, 1093, 960, 905, 687, 598; Purity 98.32 % at 300 nm, t_R= 2.837 min.

*4.1.27. 1-[(4-Methylphenyl)sulfonyl]-4-nitro-1H-benzotriazole (**23**).* Creamish Solid; yield: 92%; ^{1}H-NMR (400MHz, $CDCl_3$): δ 8.56-8.54 (d, *J* = 8.44 Hz, 1H, ArH), 8.47-8.45 (d, *J* = 7.92 Hz, 1H, Ar H), 7.65-7.61 (t, *J* = 8.04 Hz Ar H), 7.57-7.55 (d, *J* = 8.04 Hz, 2H-Tosyl), 7.15-7.13 (d, *J* = 7.96 Hz, 2H- Tosyl), 2.32 (s, 3H, CH_3); ^{13}C-NMR (100MHz, $CDCl_3$): δ 145.23, 138.69, 136.41, 132.67, 129.83, 129.04, 128.52, 127.13, 126.49, 123.26, 121.89, 117.23, 26.12; HRMS: 319.3084 [ES+]; Exact mass calcd for $C_{13}H_{10}N_4O_4S$ 318.3079 [m/z]; IR (cm^{-1}) 3283, 1543, 1481, 1328, 1134, 1093, 963, 914, 676, 574; HPLC Purity 99.71 % at 300 nm, t_R= 2.623 min.

4.2. Platelet aggregation inhibitory activity: Platelet aggregation assay was carried out using Bruker spectrophotometer. PRP (platelet rich plasma) diluted to give an absorbance unit of 0.5 apx. at 540 nm were taken in quartz cuvette. The 10 µL of ADP was added to the sample cuvette and mixed. The standard drug ticagrelor and other compounds in concentration range of 0.1 M to 0.01 nM were added to PRP before the addition of ADP. Their absorbance was monitored at every 2 minutes and IC_{50} was calculated based on aggregation of platelets after 10 minutes [27].

4.2.1. P2Y12-mediated vasodilator-stimulated phosphoprotein (VASP) phosphorylation assay: VASP phosphorylation was measured by ELISA method using a CY-QUANT VASP/P2Y12 kit. The general procedure was followed as manufacturer's recommendations. The effect of PGE1 on platelets was observed as maximum phosphorylation. The ADP was taken as standard inhibitor of phosphorylation as P2Y12 agonist. Ticagrelor was standard drug and compounds were tested at 200 µM concentration along with ADP in test solution. Furthermore, per se effect at this concentration was checked using PGE1 only without ADP on platelets.

4.3. Docking Studies: 4.3.1. Preparation of compounds: iGEMDOCK was used as molecular docking tool for carrying out molecular docking simulations. iGEMDOCK is an accurate and validated software based on GEMDOCK which uses a genetic evolutionary method for molecular docking and an empirical scoring function [36]. All compounds were prepared using ChemSketch software (ACD Labs, Canada). After that structure was cleaned and explicit hydrogens were added. Finally structure was optimized to 3D so that it can be used in iGEMDOCK. At the end structure was saved in MDL MolFile [V2000, (*.mol)] format.

4.3.2. Preparation of protein: The crystal structure of 4-NTJ at 2.62 ^{o}A resolution was retrieved from PDB (www.rscb.org). The structure contains seven transmembrane bundle of α-helices and a carboxy-terminal helix VIII that is parallel to the membrane bilayer [21]. The defined binding site was set to 'By bounded ligand' and standard parameters were set automatically by the software. "AZJ" Was Set As Binding Cite Centre. After loading the ligands, output path was set and then docking started. After completion of docking, 'view docked pose and post analysis' was done. Under interaction profile, energy of each compound was obtained and under interaction analysis interacting residues were obtained. 'Molegro Molecular Viewer' software was used to view all results.

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- Highlights
- Regioactivation by 4-nitro group in benzotriazole n-alkylation
- Alkylation under milder basic conditions
- Degradation of 4-nitrobenzotriazole under strong basic conditions
- Antiplatelet aggregation inhibitory activity
- CY QUANT VASP/P2Y12 phosphorylation assay