

Synthesis, characterization and biological activity of triazole derivatives of cinitapride

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Received 11 July 2005; accepted (revised) 22 May 2006

New triazole analogues of cinitapride are synthesized by diazotization induced hetero aromatization. Isomers resulted from the alkylation of triazole derivative are separated and one of them is synthesized in an unambiguous route. Anti-ulcer activity of all the derivatives is studied in mice.

Keywords: Cinitapride, triazole, diazotisation, methylation, prokinetic

IPC Code: Int. Cl.⁸ C07D

4-Amino-N-[1-(3-cyclohexen-1-yl-methyl)-4-piperidyl]-2-ethoxy-5-nitrobenzamide (+)-tartrate (cinitapride hydrogen tartrate) is a prokinetic benzamide derivative stimulating gastrointestinal motility and is a commercially successful anti-ulcerative active pharmaceutical ingredient^{1,2}. Reported general method for the preparation of this compound involved condensation of 4-amino-2-ethoxy-5-nitrobenzoic acid with 4-amino-1-(3-cyclohexen-1-yl-methyl)-piperidine in the presence of triethylamine and ethyl chloroformate and subsequent salt formation with L (+) tartaric acid³.

In continuation of our study of imidazole analogues of cinitapride⁴, herein we report the study of triazole analogues of cinitapride. The 1,2,3-triazole ring is a pharmacophore present in several drugs, e.g. anastrozole⁵ (antineoplastic), estazolam⁶, triazolam⁷ (sedative, hypnotic), ribavirin⁸ (antiviral), dapiprazole⁹ (antiglaucoma; miotic), cefatrizine¹⁰ (antibacterial), drometrizole¹¹ (ultraviolet-screen). The study of the impact of the presence of triazole moiety in the cinitapride skeleton on its anti-ulcerative properties is undertaken now. Synthetic chemistry of 1,2,3-benzotriazole is well documented in literature¹²⁻¹⁴. The traditional method of diazotisation induced hetero aromatisation employing sodium nitrite in acetic acid¹⁵ is used now.

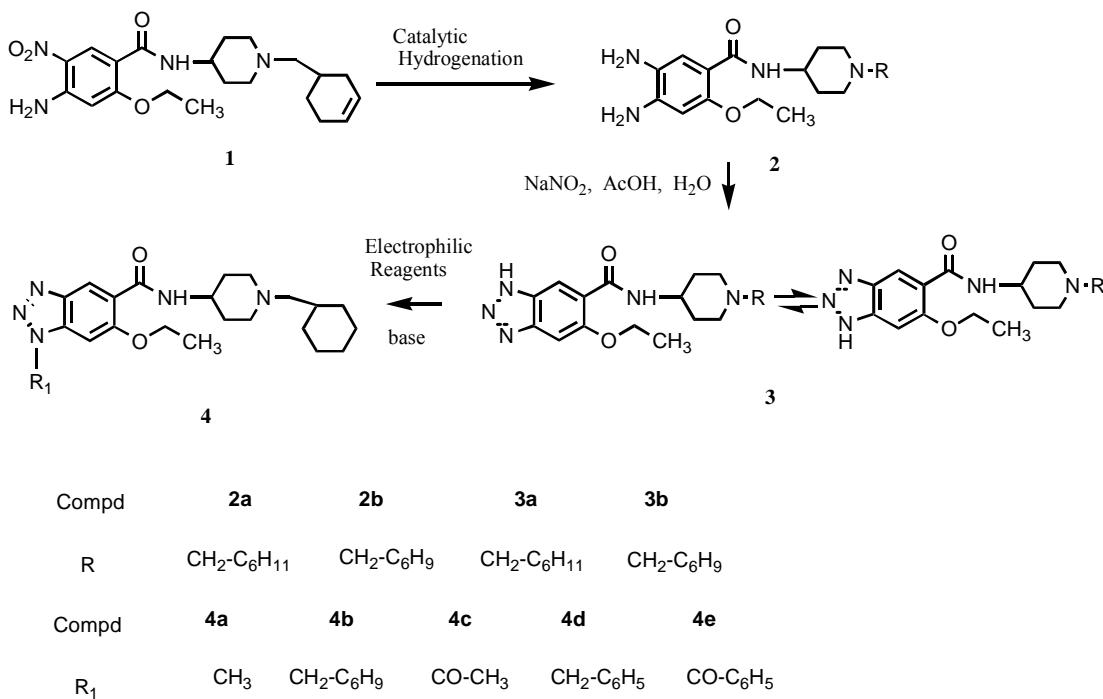
Results and Discussion

To synthesize the desired triazole derivative of cinitapride, 4-amino-N-[1-(3-cyclohexen-1-yl-methyl)-4-piperidyl]-2-ethoxy-5-nitro benzamide **1** was

chosen as the starting material. Thus, catalytic hydrogenation of **1** using Pd/C resulted in the reduction of both nitro group and cyclohexenyl double bond leading to **2a** (ref. 4). Diazotization of compound **2a** provided the corresponding triazole cinitapride derivative **3a** (**Scheme I**). IR spectrum (cm^{-1}) of compound **3a** showed a broad NH peak (3383) besides carbonyl (1655) and N=N (1623) absorptions. ^1H NMR spectrum (δ , $\text{DMSO}-d_6$) is characterized by the presence of ethoxy (1.0, t, 3H, CH_3), (4.1, q, 2H, OCH_2), triazole NH (9.8, s, 1H), aromatic (8.1, s, 1H; 9.8, s, 1H), amide NH (8.4, s, 1H) and aliphatic protons $\text{RN}-\text{CH}-\text{R}$ (3.2, m, 1H), $\text{RN}-\text{CH}_2-\text{R}$ (2.2-2.4, m, 4H), $\text{CH}_2-\text{CH}_a\text{H}_b-\text{CH}_2-\text{N}$ (1.4-1.6, m, 4H), $\text{N}-\text{CH}_2-\text{C}_6\text{H}_{11}$ (0.9-1.2, m, 11H) signals. The compound **3a** was derivatised to provide methyl, cyclohexenyl methyl, acetyl, benzyl, and benzoyl derivatives **4a-4e** by reacting with corresponding electrophilic reagents. All the compounds are fully characterized based on IR, ^1H NMR, mass spectral data and elemental analysis (**Table I**).

Raney-nickel reduction of **1** proceeded in a controlled manner with reduction of only nitro group leading to diamino derivative **2b**, which was subsequently converted into corresponding triazole derivative **3b** on treatment with $\text{NaNO}_2/\text{AcOH}$ (**Scheme I**).

Methylation of **3a** using methyl iodide provided a mixture of isomers¹⁶ in 60 : 40 ratio. This mixture was separated using silica column eluting with ethyl acetate and hexanes mixture. These isomers were



Scheme I

independently characterized based on their IR, ¹H, ¹³C NMR, 2D NMR (COSY) and mass spectral data. Further, one of these isomers, **4a** was independently synthesized by an unambiguous synthetic route starting from **1** (Scheme II). Methylation of **1** using methyl iodide and subsequent catalytic hydrogenation of resulted compound **5** yielded 5-amino-N-(1-(cyclohexylmethyl)piperidin-4-yl)-4-(cyclohexyl methylamino)-2-ethoxybenzamide **6**. Hetero-aromatization of **6** through diazotization employing sodium nitrite in acetic acid yielded compound **4a** which was found to be identical with one of the isomers obtained from direct methylation of triazole derivative **3a**, thus confirming its assigned structure in an unambiguous manner. M+1 peak at m/e 386 in mass spectrum of **4a** recorded by direct probe method indicated the formation of desired product. It was further confirmed by IR and ¹H NMR spectral data and also from elemental analysis.

Gastric emptying studies in Swiss albino mice

Prokinetic studies were conducted in Preclinical Biology Department, Discovery Research, Dr Reddy's Laboratories Ltd, Hyderabad. Compounds were tested on male Swiss albino mice (body weight 20-25 g) at dosage of 10 mg / kg orally.

Methodology: Adult Swiss male albino mice weighing between 20-25 g were used for our study.

Mice were fasted overnight prior to experimentation but had free access to water. Phenol red (0.5 mL) meal was administered orally 1 hr after the drug administration. Animals were sacrificed by cervical dislocation 15 min after the administration of the meal. Abdomen was opened and stomach was dissected out. The stomach was cut into pieces and homogenized with 10 mL of 0.1 N NaOH. To this 1.9 mL of homogenate and 0.19 mL of trichloroacetic acid (20% w/v) were added and centrifuged at 3000 rpm for 20 min. To 1.5 mL of supernatant, 0.5 mL of 0.5 N NaOH was added and absorbance was measured at 560 nm. This correlates to the concentration of phenol red in the stomach, which in turn depends on gastric emptying (GE).

$$\% \text{ GE} = (1 - X/Y) * 100$$

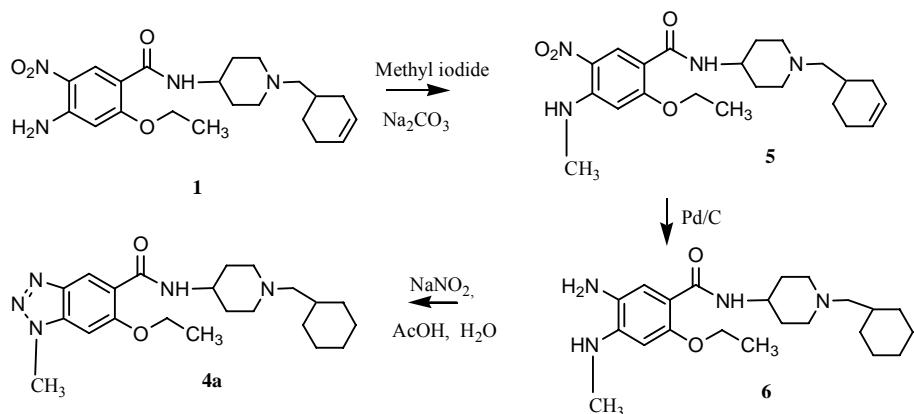
X-----Absorbance of phenol red recovered after 15 min after test meal.

Y-----Absorbance of phenol red recovered at 0 min following test meal.

Results: Compounds **2b** and **5** showed significant acceleration of gastric emptying in mice. Cinitapride was taken as our reference standard.

Table I — Characterization data of compounds

Compd	Yield (%)	m.p.	¹ H NMR (DMSO- <i>d</i> ₆ , <i>δ</i>)	Mass (M+1)	Mol. Formula	CHN Analysis		
						Calculated% (found)		
						C	H	N
2b	91.8	177-79	1.0 (t, 3H, CH, <i>J</i> =7.0 Hz), 1.2-1.3 (m, 3H, CH), 1.4-1.6 (m, 4H, CH), 2.2 (m, 4H, CH), 2.2 (m, 4H, CH), 2.4 (m, 2H, CH), 3.2 (m, 1H, CH), 4.1 (q, 2H, CH, <i>J</i> =7.06 Hz), 4.6-4.8 (m, 1H, CH), 5.6-5.8 (m, 1H, CH), 7.2 (s, 1H, ArH), 8.1 (s, 1H, ArH), 8.4 (s, 1H, NH)	373	C ₂₁ H ₃₂ N ₄ O ₂	67.71 (67.64)	8.66 8.60	15.04 15.01)
3a	82.6	195-98	1.0 (t, 3H, CH, <i>J</i> =7.0 Hz), 0.9-1.2 (m, 11H, N-CH ₂ -C ₆ H ₁₁), 1.4-1.6 (m, 4H, CH), 2.2 (m, 4H, CH), 2.4 (m, 2H, CH), 3.2 (m, 1H, CH), 4.1 (q, 2H, CH, <i>J</i> =7.06 Hz), 7.2 (s, 1H, ArH), 8.1 (s, 1H, ArH), 8.4 (s, 1H, N-H), 9.8 (b, 1H, N-H in triazole)	386	C ₂₁ H ₃₁ N ₅ O ₂	65.43 (65.37)	8.11 8.03	18.17 18.11)
3b	80.2	168-72	1.0 (t, 3H, CH, <i>J</i> =7.0 Hz), 1.2-1.3 (m, 3H, CH), 1.4-1.6 (m, 4H, CH), 2.1 (m, 4H, CH), 2.2 (m, 4H, CH), 2.4 (m, 2H, CH), 3.2 (m, 1H, CH), 4.1 (q, 2H, CH, <i>J</i> =7.06 Hz), 4.6-4.8 (m, 1H, CH), 5.6-5.8 (m, 1H, CH), 7.2 (s, 1H, ArH), 8.1 (s, 1H, ArH), 8.4 (s, 1H, N-H), 9.8 (b, 1H, N-H in triazole)	384	C ₂₁ H ₂₉ N ₅ O ₂	65.77 (65.66)	7.62 7.58	18.26 18.20)
4a	45.8	185-88	1.0 (t, 3H, CH, <i>J</i> =7.0 Hz), 0.9-1.2 (m, 11H, N-CH ₂ -C ₆ H ₁₁), 1.4-1.6 (m, 4H, CH), 2.2 (m, 4H, CH), 2.3 (s, 3H, CH), 2.4 (m, 2H, CH), 3.2 (m, 1H, CH), 4.1 (q, 2H, CH, <i>J</i> =7.06 Hz), 7.2 (s, 1H, ArH), 8.1 (s, 1H, ArH), 8.4 (s, 1H, N-H)	400	C ₂₂ H ₃₃ N ₅ O ₂	66.14 (66.10)	8.33 8.28	17.53 17.49)
4b	39.8	202-05	1.0 (t, 3H, CH, <i>J</i> =7.0 Hz), 0.9-1.2 (m, 11H, N-CH ₂ -C ₆ H ₁₁), 1.2-1.3 (m, 3H, CH); 1.4-1.6 (m, 4H, CH), 2.1 (m, 4H, CH), 2.2 (m, 4H, CH), 2.4 (m, 4H, CH), 3.2 (m, 1H, CH), 4.1 (q, 2H, CH, <i>J</i> =7.06 Hz), 4.6-4.8 (m, 1H, CH), 5.6-5.8 (m, 1H, CH), 7.2 (s, 1H, ArH), 8.1 (s, 1H, ArH), 8.4 (s, 1H, N-H)	480	C ₂₈ H ₄₁ N ₅ O ₂	70.11 (70.70)	8.62 8.57	14.60 14.56)
4c	41.6	202-05	1.02 (t, 3H, CH, <i>J</i> =7.0 Hz), 0.9-1.2 (m, 11H, N-CH ₂ -C ₆ H ₁₁), 1.4-1.6 (m, 4H, CH), 2.2 (m, 4H, CH), 2.4 (m, 2H, CH), 2.6 (s, 3H, CH), 3.2 (m, 1H, CH), 4.1 (q, 2H, CH, <i>J</i> =7.06 Hz), 7.2 (s, 1H, ArH), 8.1 (s, 1H, ArH), 8.4 (s, 1H, N-H)	428	C ₂₃ H ₃₃ N ₅ O ₃	64.61 (64.57)	7.78 7.70	16.38 16.32)
4d	43.5	165-69	1.0 (t, 3H, CH, <i>J</i> =7.0 Hz), 0.9-1.2 (m, 11H, N-CH ₂ -C ₆ H ₁₁), 1.4-1.6 (m, 4H, CH), 2.2 (m, 4H, CH), 2.4 (m, 2H, CH), 2.6 (m, 2H, CH), 3.2 (m, 1H, CH), 4.1 (q, 2H, CH, <i>J</i> =7.06 Hz), 7.2 (s, 6H, ArH), 8.1 (s, 1H, ArH), 8.4 (s, 1H, N-H)	476	C ₂₈ H ₃₇ N ₅ O ₂	70.71 (70.67)	7.84 7.80	14.72 14.68)
4e	39.4	189-93	¹ H NMR (DMSO): <i>δ</i> , 1.0 (t, 3H, CH, <i>J</i> =7.0 Hz), 0.9-1.2 (m, 11H, N-CH ₂ -C ₆ H ₁₁), 1.4-1.6 (m, 4H, CH), 2.2 (m, 4H, CH), 2.4 (m, 2H, CH), 3.2 (m, 1H, CH), 4.1 (q, 2H, CH, <i>J</i> =7.06 Hz), 7.3 (s, 6H, ArH), 8.1 (s, 1H, ArH), 8.4 (s, 1H, N-H)	490	C ₂₈ H ₃₅ N ₅ O ₃	68.69 (68.60)	7.21 7.17	14.30 14.27)
5	70.0	165-68	1.0 (t, 3H, CH, <i>J</i> =7.0 Hz), 1.2-1.3 (m, 3H, CH), 1.4-1.6 (m, 4H, CH), 2.2 (m, 4H, CH), 2.3 (s, 3H, CH), 2.4 (m, 6H, CH), 3.2 (m, 1H, CH), 4.1 (q, 2H, CH, <i>J</i> =7.06 Hz), 4.6-4.8 (m, 1H, CH), 5.6-5.8 (m, 1H, CH), 7.2 (s, 1H, ArH), 8.1 (s, 1H, Ar)	417	C ₂₂ H ₃₂ N ₄ O ₄	63.44 (63.35)	7.74 7.68	13.45 3.4)
6	90.1	163-72	1.02 (t, 3H, CH, <i>J</i> =7.0 Hz), 0.9-1.2 (m, 11H, N-CH ₂ -C ₆ H ₁₁), 1.4-1.6 (m, 4H, CH), 2.2 (m, 4H, CH), 2.4 (m, 2H, CH), 2.6 (m, 3H, CH), 3.2 (m, 1H, CH), 4.1 (q, 2H, CH, <i>J</i> =7.06 Hz), 7.2 (s, 1H, ArH), 8.1 (s, 1H, ArH)	389	C ₂₂ H ₃₆ N ₄ O ₂	68.01 (68.0)	9.34 9.27	14.42 14.38)



Scheme II

Table II — Gastric emptying study of compounds

Treatment	Control	2b	3a	3b	4a	4b	4c	4d	4e	5	6
dose/kg	10	10	10	10	10	10	10	10	10	10	10
% GE	66 ± 3	78 ± 4*	55 ± 5	66 ± 6	58 ± 6	65 ± 2	77 ± 4	60 ± 4	55 ± 1	75 ± 1	58 ± 5*

Experimental Section

Melting points were determined on a Thomas-Hoover capillary. IR spectra were recorded on a Nicolet 550 Series II Maguna FT-IR spectrometer. ¹H NMR spectra were measured on a Varian Gemini 200 MHz spectrometer in DMSO-*d*₆ with TMS as an internal standard. Micro analyses were performed for C, H, N (Dr Reddy's Research Foundation, Hyderabad) and were within ±0.4% of theoretical values. Silica gel column chromatography was performed using Merck silica gel 60 ASTM (70-230) mesh.

Synthesis of 4,5-diamino-N-(1-((cyclohex-3-enyl)methyl)piperidin-4-yl)-2-ethoxybenzamide 2b. To a solution of *ortho*-nitrophenylamine derivative (**1**, 20 g, 0.049 mole) in methanol (300 mL) in a hydrogen autoclave, Raney-Ni (4 g) was added and 3 kg H₂↑ pressure was applied under stirring. Reaction mixture was maintained up to 3 hr and hydrogen pressure was released. Catalyst was filtered, washed with methanol (75 mL), organic layer was distilled at 50°C under vacuum. The residue was stirred in ethyl acetate (250 mL) filtered and washed with ethyl acetate (50 mL) to afford compound **2b**.

Synthesis of N-(1-(cyclohexylmethyl)piperidin-4-yl)-6-ethoxy-1H-benzo[d][1,2,3]triazole-5-carboxamide 3a and N-(1-((cyclohex-3-enyl)methyl)piperidin-4-yl)-6-ethoxy-1H-benzo[d][1,2,3]triazole-5-carboxamide 4a. To a solution of *N*-(1-(cyclohexylmethyl)piperidin-4-yl)-6-ethoxy-1-methyl-1*H*-benzo[d][1,2,3]triazole-5-carboxamide **3a** (3 g, 0.0077 mole) in dimethyl formamide (15 mL), was added Na₂CO₃ (0.8 g) and electrophilic reagent (0.0083 mole) in dimethyl formamide (15 mL). Resulting solution was stirred for 10 hr at 30°C. Reaction mass was quenched with water (30 mL) and insoluble material removed by filtration. Reaction mass was extracted with ethylacetate 60 mL. Organic phase was washed with water and solvent was completely removed under vacuum. Compound was purified by column chromatography using ethyl acetate and hexanes (1:1) mixture as eluent.

din-4-yl)-6-ethoxy-1*H*-benzo[d][1,2,3]triazole-5-carboxamide **3b.** To a solution of 1,2-diamino compound (**2a/2b** 0.026 mole) in acetic acid (10 mL, 0.16 mole) and water (50 mL), was added aqueous NaNO₂ solution (2 g, 20 mL, 0.028 mole) at 5°C for 0.5 hr. Resulting solution was stirred for 1 hr at 5°C and an additional period of 1 hr at 45°C. Solid was filtered and washed with water (10 mL) at 25°C to afford triazole compound **3a/3b**.

Synthesis of *N*-(1-(cyclohexylmethyl)piperidin-4-yl)-6-ethoxy-1-methyl-1*H*-benzo[d][1,2,3]triazole-5-carboxamide **4a and 1-((cyclohex-3-enyl)methyl)-*N*-(1-(cyclohexylmethyl)piperidin-4-yl)-6-ethoxy-1*H*-benzo[d][1,2,3]triazole-5-carboxamide **4b**.** To a solution of *N*-(1-(cyclohexylmethyl)piperidin-4-yl)-6-ethoxy-1-methyl-1*H*-benzo[d][1,2,3]triazole-5-carboxamide **3a** (3 g, 0.0077 mole) in dimethyl formamide (15 mL), was added Na₂CO₃ (0.8 g) and electrophilic reagent (0.0083 mole) in dimethyl formamide (15 mL). Resulting solution was stirred for 10 hr at 30°C. Reaction mass was quenched with water (30 mL) and insoluble material removed by filtration. Reaction mass was extracted with ethylacetate 60 mL. Organic phase was washed with water and solvent was completely removed under vacuum. Compound was purified by column chromatography using ethyl acetate and hexanes (1:1) mixture as eluent.

Synthesis of 1-acetyl-N-(1-(cyclohexylmethyl)piperidin-4-yl)-6-ethoxy-1H-benzo[d][1,2,3]triazole-5-carboxamide 4c, 1-benzyl-N-(1-(cyclohexylmethyl)piperidin-4-yl)-6-ethoxy-1H-benzo[d][1,2,3]triazole-5-carboxamide 4d and 1-benzoyl-N-(1-(cyclohexylmethyl)piperidin-4-yl)-6-ethoxy-1H-benzo[d][1,2,3]triazole-5-carboxamide 4e. To a solution of *N*-(1-(cyclohexylmethyl)piperidin-4-yl)-6-ethoxy-1H-benzo[d][1,2,3]triazole-5-carboxamide **3a** (3 g, 0.0077 mole) in chloroform (15 mL), was added pyridine (0.1 mL) and electrophilic reagent (0.0083 mole) in chloroform. Resulting solution was stirred for 4 hr at 40°C. Reaction mass was quenched with water (30 mL) and organic layer was separated. Separated layer was washed with saturated sodium bicarbonate solution followed by water and solvent was removed under vacuum. Resulted compound was purified by column chromatography using ethyl acetate and hexanes (1:1) mixture as eluent.

Synthesis of *N*-(1-((cyclohex-3-enyl)methyl)piperidin-4-yl)-2-ethoxy-4-(methylamino)-5-nitrobenzamide 5. To a solution of **1** (10 g, 0.024 mole) in dimethyl formamide (50 mL), was added Na₂CO₃ (3.25 g, 0.031 mole) and methyliodide (3.75 g, 0.026 mole) in dimethyl formamide (10 mL). Resulting solution was stirred for 4 hr at 30°C. Reaction mass was quenched with water 100 mL. Insoluble material was removed by filtration. Reaction mass was extracted with ethylacetate (3 × 250 mL). Organic phase was washed with water and solvent was completely removed under vacuum. Compound was purified by column chromatography using ethylacetate and hexanes (1:1) mixture as eluent.

Synthesis of 5-amino-N-(1-(cyclohexylmethyl)piperidin-4-yl)-2-ethoxy-4-(methylamino) benzamide 6. To a solution of **5** (2.0 g, 0.0048 mole) in methanol (200 mL) in a hydrogen autoclave, Pd in carbon (5%, 0.25 g) was added and 3 kg H₂↑ pressure was applied under stirring. Reaction mixture was maintained up to 2 hr and hydrogen pressure was released. Catalyst was filtered, washed with methanol and methanolic solution was distilled at 50°C, filtered and washed with ethyl acetate (50 mL) to provide diamino compound **6**.

Synthesis of *N*-(1-(cyclohexylmethyl)piperidin-4-yl)-6-ethoxy-1-methyl-1H-benzo[d][1,2,3]triazole-5-carboxamide 4a. To a solution of diamino compound **6** (2 g, 0.005 mole) in acetic acid (3 mL) and water (6 mL), was added aqueous sodium nitrite solution (0.45 g, 5 mL, 0.006 mole) at 0-5°C for 0.5 hr. Resulting solution was stirred for 1 hr at 5°C and one more hour at 45°C. Compound **4a** was filtered and purified by column chromatography using ethylacetate and hexanes mixture (1:1) as eluent.

Acknowledgement

The authors wish to thank the management of Dr Reddy's Laboratories Ltd., Bulk Actives Unit-III, Discovery Research, Dr Reddy's Laboratories Ltd and Mr S. Venkatraman, Sr Vice President, R&D for supporting this work.

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