

Synthesis and Characterization of Impurities of an Broad- Spectrum Anthelmintic Drug, Albendazole

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Abstract: Albendazole (**1**) is a broad-spectrum anthelmintics drug. Albendazole impurity A, 5-(propylthio)-1*H*-benzimidazol-2-amine (**6**), impurity B, methyl [5-(propylsulfinyl)- 1*H*-benzimidazol-2-yl]carbamate (**7**), impurity C, methyl [5-(propylsulfonyl)-1*H*- benzimidazol-2-yl]carbamate (**8**) and impurity D, 5-(propylsulfonyl)-1*H*- benzimidazol-2-amine (**9**) are degradation or metabolic impurities whereas impurity E, methyl 1*H*-benzimidazol- 2-yl]carbamate (**11**) and impurity F, methyl [5-(methylthio)-1*H*- benzimidazol-2-yl]carbamate (**15**) are process related impurities. All these impurities are listed in European pharmacopoeia. Present work describes the synthesis and characterization of all these six impurities.

Keywords: Albendazole, Impurities, Synthesis, Characterization

Introduction

Albendazole is a broad-spectrum anthelmintics against gastrointestinal nematodes and the larval stages of cestodes¹. Recently, the anticancer effects of albendazole were also investigated^{2,3}. The principal mode of action for Albendazole is by its inhibitory effect on tubulin polymerization which results in the loss of cytoplasmic microtubules. Albendazole was first discovered at the SmithKline Animal Health Laboratories in 1972.

Impurities can be closely related to the product that is formed during the synthesis of a bulk drug or it can be decomposition product formed during the storage of the drug. The presence of impurities in an active pharmaceutical ingredient (API) can have a significant impact on the quality and safety of the drug products. Therefore, it is necessary to study the impurity profile of the API to be used in the manufacturing of drug substance. International conference on harmonization (ICH) guidelines recommended identifying and characterizing all impurities that are present at a level of 0.10% or more^{4,5}.

Albendazole is from benzimidazole carbamate family chemically known as methyl-[(6-propylthio)-1*H*-benzimidazol-2-yl]carbamate (**1**). Albendazole synthesis using various methods

is already disclosed^{6,7}. Recently efficient process for albendazole preparation at scale is reported⁸. Various albendazole analogues were synthesized and evaluated for their biological activity^{9,10}.

Experimental

The IR spectra were recorded in the solid state as using Shimadzu IR Affinity-1 spectrophotometer. ¹H NMR spectra were recorded on Bruker Advance III 400 MHz spectrometer, the chemical shifts are reported in δ ppm relative to TMS. The mass spectra were recorded on LCMS- API-2000, ABSciex. Albendazole API was synthesized as per literature method⁸. Reaction conditions mentioned here are not optimized and yields are of isolated and purified products.

5-(Propylthio)-1H-benzimidazol-2-amine (6)

Reaction mixture consisting solution of albendazole (**1**, 3.0 g, 11.3 mmole) in concentrated sulphuric acid: water (30 mL, 1:1; v/v) was heated to reflux temperature (100 °C) maintained while stirring for 16 h at same temperature. Reaction progress was monitored by TLC (100%, ethyl acetate, R_f = 0.1). Reaction mass was then cooled to room temperature (23-25 °C) and poured into ice cold water (100 mL). Reaction mass was basified using aqueous sodium hydroxide solution (10%, 200 mL). Reaction mixture was extracted using ethyl acetate (3×50 mL), organic layers were combined dried over sodium sulfate, filtered and concentrated under reduced pressure to get crude product residue. Crude product residue was purified by silica gel column chromatography to get buff colored solid, yield 28%, 0.65 g, HPLC purity 89.80%, mp 154.6 °C; IR (ν_{\max} , cm^{-1}) 3388 (N-H), 2960-2872 (aliphatic-H), 1651-1645 (C=C stretching), 1556 (C-C stretching ring), 1016, 885, 748 (C-H bending). ¹H NMR (DMSO- d_6) 10.69 (1H, bs, NH), 7.05-7.048 (1H_{arom}, d), 6.97-6.94 (1H_{arom}, d), 6.85-6.81 (1H_{arom}, dd), 6.2 (2H, bs, NH₂), 2.75-2.65 (2H, t, -CH₂), 1.46-1.39 (2H, q, -CH₂CH₂CH₃), 0.90-0.84 (3H, t, -CH₃). MS, m/z 208 [M+H]⁺.

Methyl [5-(propylsulfinyl)-1H-benzimidazol-2-yl]carbamate (7)

To a solution of Albendazole (**1**, 3.0 g, 11.3 mmole) in methanol: water (30 mL; 1:1; v/v) was added oxone (1.72 g; 11.3 mmole) in portions at 5-10 °C. Reaction mass was then warmed to room temperature, stirred for 14 h. Solid obtained was filtered, washed with water (20 mL). Filtrate was concentrated under reduced pressure to get crude product was dissolved in methanol in dichloromethane (20 mL, 1:9; v/v). Solid formation was observed was filtered and filtrate was concentrated under reduced pressure to get off-white colored solid, yield 28%, 0.90 g, HPLC purity 95.35%, mp 195.1 °C; IR (ν_{\max} , cm^{-1}) 3223 (N-H), 2958-2866 (aliphatic-H), 1755 (amide N-H), 1651-1633 (C=C stretching), 1467 (C-H stretching), 1016, 885, 748 (C-H bending). ¹H-NMR (DMSO- d_6) 7.8 (1H_{arom}, s), 7.7 (1H_{arom}, d), 7.5 (1H_{arom}, d), 3.9 (3H, s, -OCH₃), 3.7-3.8 (2H, t, -SCH₂), 1.5-1.6 (2H, m, -CCH₂), 0.9 (3H, t, -CH₃). MS, m/z 282 [M+H]⁺.

Methyl [5-(propylsulfonyl)-1H-benzimidazol-2-yl]carbamate (8)

To a solution of Albendazole (**1**, 6.0 g, 22.6 mmole) in dichloromethane (100 mL) was added *m*-chloroperbenzoic acid (7.8 g, 45.3 mmole) in portions at 5-10 °C. Reaction mass was then warmed to room temperature (23-25 °C), stirred for 9 h. Reaction progress was monitored by TLC (petroleum ether:ethyl acetate, 1:1, R_f = 0.12). Reaction mass was concentrated under reduced pressure to get residue as crude product. Crude product was purified on silica gel column chromatography to get off-white solid, yield 97%, 6.50 g, HPLC

purity 93.94%, mp 282.6 °C; IR (ν_{\max} , cm^{-1}) 3234 (N-H), 2958-2866 (aliphatic-H), 1697 (amide N-H), 1651-1643 (C=C stretching), 1595-1427 (stretching in ring), 1271-1107 (SO stretching). ^1H NMR (DMSO- d_6) 12.35 (1H, bs, -NH), 11.64 (1H, bs, -NH), 7.9 (1H_{arom}, s), 7.6 (2H_{arom}, dd), 3.8 (3H, s, -OCH₃), 3.25 (2H, t, -SCH₂), 1.5 (2H, m, -CCH₂), 0.85 (3H, t, -CH₃). MS, m/z 298 [M+H]⁺.

5-(Propylsulphonyl)-1H-benzimidazol-2-amine (9)

Reaction mixture consisting solution of (8) (2.5 g, 8.4 mmole) in concentrated sulphuric acid: water (30 mL, 1:1; v/v) was heated to reflux temperature (100 °C) maintain while stirring for 13 h at same temperature. Reaction progress was monitored by TLC (100%, ethyl acetate, $R_f=0.13$). Reaction mass was cooled to room temperature and poured on ice-cold water (100 mL). Reaction mass was washed with ethyl acetate (2×50 mL) to remove non-polar impurities. Reaction mass was basified using aqueous sodium hydroxide solution (10%, 200 mL) and extracted using ethyl acetate (3×50 mL), organic layers were combined dried over sodium sulfate, filtered and concentrated under reduced pressure to get orange colored solid (hygroscopic), yield 39%, 0.78 g, HPLC purity 96.68%; IR (ν_{\max} , cm^{-1}) 3344 (N-H), 2958-2866 (aliphatic-H), 1651-1643 (N-H), 1427-1276 (SO stretching). ^1H NMR (DMSO- d_6) 11.2 (1H, bs, indole-NH), 7.5 (1H_{arom}, s), 7.35 (1H_{arom}, d), 7.25 (1H_{arom}, d), 6.7 (2H, s, NH₂), 3.15 (2H, t, -SCH₂), 1.5 (2H, m, -CCH₂), 0.8-0.9 (3H, t, -CH₃). MS, m/z 240 [M+H]⁺, 262 [M+Na].

Methyl 1H-benzoimidazol-2-yl carbamate (11)

To a solution of 2-aminobenzimidazole (10) (2.0 g, 15.0 mmole) in acetone (30 mL) was added triethylamine (1.8 g, 18.0 mmole) at room temperature, stirred for 15 min. To above reaction mass was added methyl chloroformate (1.7 g, 17.0 mmole) drop wise at room temperature, reaction mass was stirred for 10 h at same temperature. Reaction was monitored by TLC (50% ethyl acetate in petroleum ether, $R_f=0.65$). Solid appeared was filtered off, washed with little acetone (50 mL) then with water (20 mL). Solid dried under vacuum to get white colored solid, yield 35%, 1.50 g, HPLC purity 99.59%, mp 168.2 °C; IR (ν_{\max} , cm^{-1}) 3442 (N-H), 2601-2497 (aliphatic-H), 1722 (amide N-H), 1647 (C=C stretching), 1456, 1367 (aliphatic-H bending), 1215 (C-O-C asymmetric stretching), 765, 748 (C-H bending). ^1H NMR (DMSO- d_6): 7.95-7.90 (1H_{arom}, m), 7.15-7.04 (2H_{arom}, m), 7.18 (1H, s, indole-NH), 7.55 (1H_{arom}, d), 3.95 (3H, s, -OCH₃). MS, m/z 192 [M+H]⁺.

Methyl [5-(methylthio)-1H-benzimidazol-2-yl]carbamate(15)

Step-I: 2-Nitro-4-thiocyanoaniline (3)

To a mixture of 2-nitroaniline (5.0 g, 36.2 mmole) and ammonium thiocyanate (5.65 g, 74.2 mmole) in methanol (40 mL) was purged chlorine gas (generated in laboratory using potassium permanganate and hydrochloric acid) for 6 h at room temperature, stirred for 2 h at same temperature. Reaction progress was monitored by TLC (20% ethyl acetate in petroleum ether, $R_f=0.4$). To reaction mass was added water (50 mL), stirred at 20 °C for 1 h. Solid was filtered, washed with water (20 mL) and dried under vacuum to give yellow colored solid (3), yield 59%, 4.2 g. This was used as such for the next step.

Step-II: 4-(Methylthio)-2-nitroaniline (12)

To a solution of (3) (4.0 g, 20.5 mmole) in methanol:water (20 mL, 1:1, v/v) was added sodium hydroxide (3.44 g, 86.0 mmole) at room temperature. Reaction mass then heated to 40 °C, stirred at same temperature for 1 h. Reaction mass was cooled to 10-15 °C was added

methyl iodide (2.61 mL, 41.0 mmole) at 10-15 °C. Reaction mass was heated to 40 °C and stirred for 4 h. Reaction progress was monitored by TLC (20% ethyl acetate in petroleum ether, $R_f=0.65$). Reaction mass cooled to room temperature, concentrated under reduced pressure to remove methanol completely. To reaction mass was added water (50 mL) extracted using ethyl acetate (3×20 mL). Combined organic layers dried over sodium sulfate, filtered and concentrated under reduced pressure to get orange colored solid, yield 72%, 2.70 g. This was used as such for the next step.

Step- III: 4-(Methylthio)benzene-1,2-diamine (13)

To a solution of (12) (2.7 g, 14.7 mmole) in ethanol (25.0 mL) was added concentrated hydrochloric acid (2.0 mL) at 10-15 °C was stirred for 10 min. Reaction mass warmed to room temperature was added iron (4.1 g 73.4 mmole), stirred at room temperature for 4 h. Reaction progress was monitored by TLC (20% ethyl acetate in petroleum ether, $R_f=0.5$). To the reaction mass was added ethyl acetate: water (100 mL, 1:1; v/v), basify using sodium hydroxide pellets (4.6 g). Reaction mass was filtered through hi-flo for better separation of organic layer from aqueous. Organic layer separated dried over sodium sulfate, filtered and concentrated under reduced pressure to get brown colored solid, yield 89%, 2.0 g. This was used as such for the next step.

Step-IV: Methyl-N-cyanocarbamate sodium salt (14)

To a solution of cyanamide (4.0 g, 95.2 mmole) in water (15 mL) was added methyl chloroformate (6.0 g, 63.5 mmole) and sodium hydroxide (5.5 g, 137.5 mmole) was added simultaneously while maintaining temperature below 10 °C and pH 7-7.5. After addition was complete pH was adjusted to 8-8.5 using aqueous sodium hydroxide solution. Reaction mass was maintained for 2 h at 10-15 °C. Reaction mass evaporated under reduced pressure to get white solid, yield 100%, 11.61 g. This reagent was used as such for next step.

Step-V: Methyl [5-(methylthio)-1H-benzimidazole-2-yl]carbamate (15)

To a solution of (13) (2.0 g, 12.9 mmole) in mixture of acetone (20 mL) and water (2 mL) was added concentrated hydrochloric acid (5 mL) at room temperature. To the reaction mass was added (11) (11.61 g, 95.1 mmole) at room temperature. Reaction mass was then heated at 80-85 °C, maintained for 3 h. Reaction progress was monitored by TLC (80% ethyl acetate in petroleum ether, $R_f=0.7$). Reaction mass was acidified using concentrated hydrochloric acid to pH 4.0-4.5. Solid obtained was filtered, washed with water (50 mL) and ethyl acetate (10 mL). Blue colored solid was dried under reduced pressure, yield 52%, 1.60 g, HPLC purity 92.74%, mp 147.2 °C. IR (ν_{\max} , cm^{-1}) 3323-3200 (N-H), 2948-2800 (aliphatic-H), 1737 amide N-H), 1627-1604 (C=C stretching), 1249 (C-S stretching), 800, 765, 748 (C-H bending). MS: m/z 238 [M+H]⁺, 260 [M+Na], 236 [M-H]⁻. ¹H-NMR (DMSO- d_6): 7.30-7.25 (1H_{arom}, dd), 7.45 (1H_{arom}, d), 7.55-7.5 (1H_{arom}, d), 3.8 (3H, s, -OCH₃), 2.5 (3H, s, -SCH₃).

Results and Discussion

All six Albendazole impurities as per EP monograph are needed to synthesize individually in pure form. Scope of current work was to synthesize these impurities. Albendazole impurity A; 5-(propylthio)-1H-benzimidazol-2-amine (6), impurity B; methyl [5-(propylsulfinyl)-1H-benzimidazol-2-yl]carbamate (7), impurity C; methyl [5-(propylsulfonyl)-1H-benzimidazol-2-yl]carbamate (8) and impurity D; 5-(propylsulfonyl)-1H-benzimidazol-2-amine (9) are degradation or metabolic impurities (Figure 1).

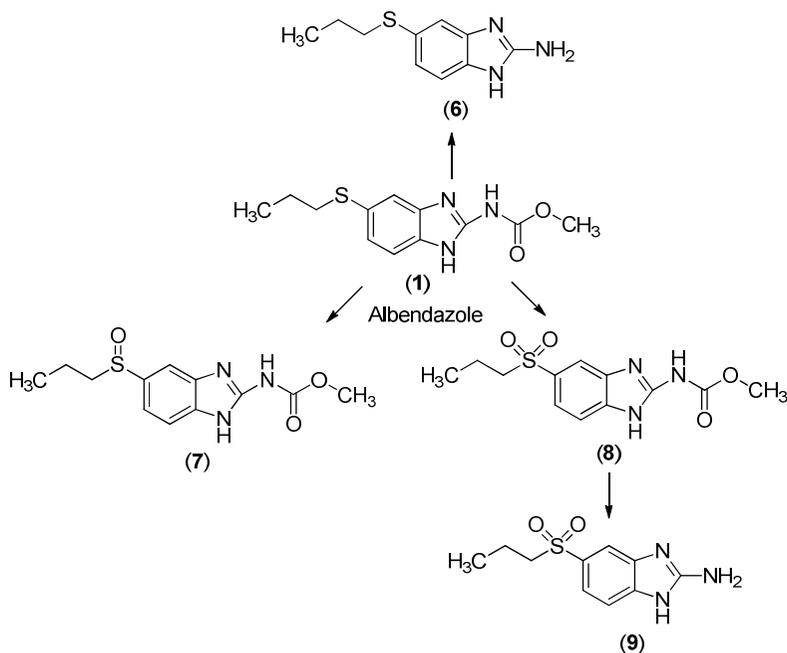
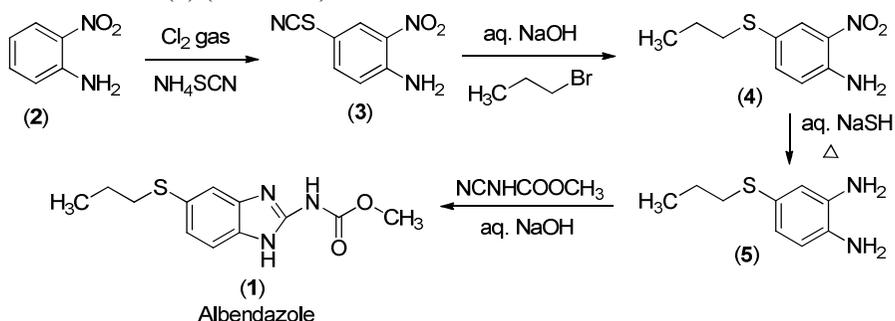


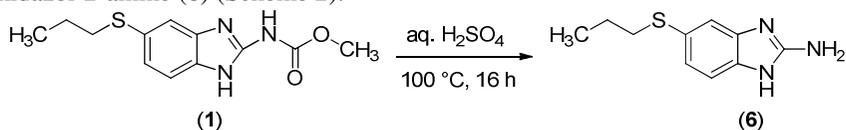
Figure 1. Degradation or metabolic impurities

Albendazole (**1**) was synthesized as reported in literature⁸ from 2-nitroaniline (**2**) in four steps, first step involves thiocyanation followed by *S*-alkylation using *n*-propyl bromide under basic condition gives 4-(propylthio)-2-nitroaniline (**4**). Reduction of compound (**4**) using sodium hydrosulfide on treatment with methyl-*N*-cyano carbamate yields albendazole (**1**) (Scheme 1).



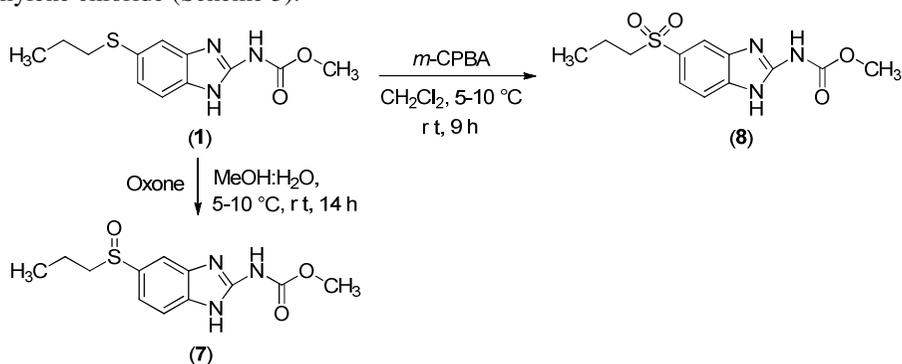
Scheme 1

Albendazole was subjected to hydrolysis condition using aqueous sulfuric acid at reflux temperature to get corresponding amine as impurity A (EP); 5-(propylthio)-1*H*-benzimidazol-2-amine (**6**) (Scheme 2).



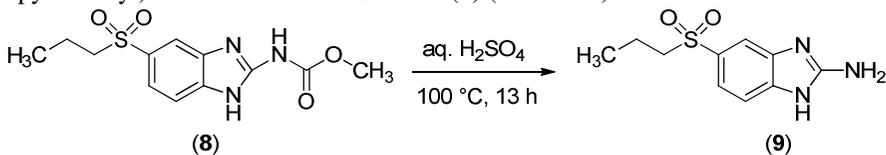
Scheme 2

Albendazole rapidly converted in the liver to the primary metabolite, albendazole sulfoxide, which is further metabolized to albendazole sulfone and other primary oxidative metabolites that have been identified in human urine^{11,12}. Albendazole sulfoxide and sulfone, oxidative degradation impurities or oxidative metabolites are listed as impurity B; methyl [5-(propylsulfonyl)-1*H*-benzimidazol-2-yl]carbamate (**7**) and impurity C; methyl [5-(propylsulfonyl)-1*H*-benzimidazol-2-yl]carbamate (**8**) respectively in EP monograph. Albendazole sulfoxide was obtained by oxidation of albendazole using Oxone in aqueous methanol and sulfone using oxidation with *meta*-chloroperbenzoic acid (*m*-CPBA) in methylene chloride (Scheme 3).



Scheme 3

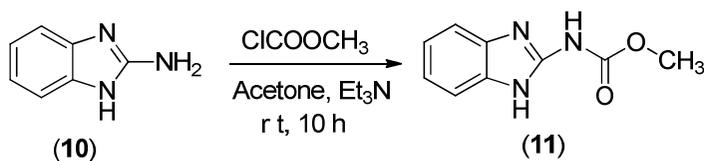
Methyl [5-(propylsulfonyl)-1*H*-benzimidazol-2-yl]carbamate (**8**) was obtained from Albendazole oxidation as impurity C was hydrolyzed under acidic condition using aqueous sulfuric acid at high temperature to get corresponding amine as impurity D, 5-(propylsulfonyl)-1*H*-benzimidazol-2-amine (**9**) (Scheme 4).



Scheme 4

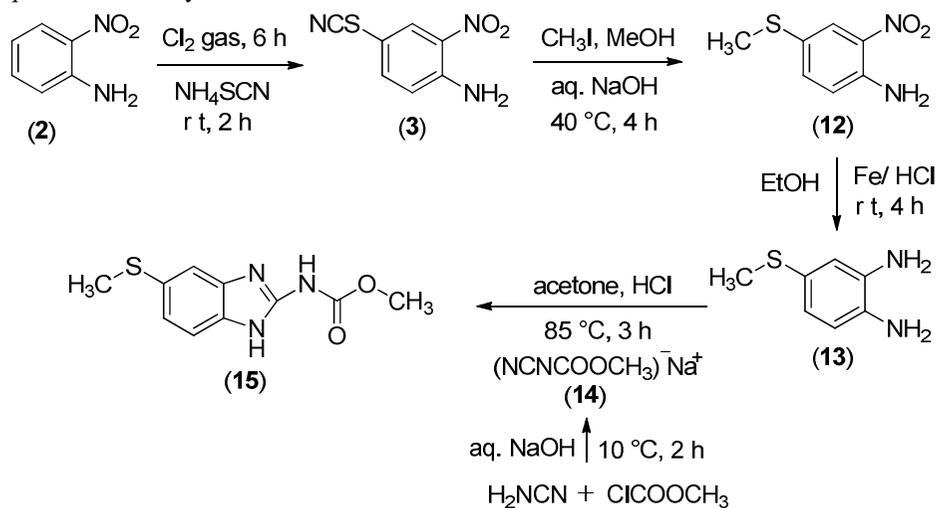
Albendazole impurity E, methyl 1*H*-benzimidazol-2-yl]carbamate (**11**) and impurity F, methyl [5-(methylthio)-1*H*-benzimidazol-2-yl]carbamate (**15**) are process related impurities. Formation of impurity E (**11**) in API synthesis came from unreacted 2-nitroaniline (**2**) in thiocyanation reaction carried forward till end. Impurity F (**15**) is side reaction due to *S*-methylation instead of *n*-propylation in API synthesis. This may be because of methyl halide contamination in *n*-propyl halide.

Albendazole impurity E, methyl 1*H*-benzimidazol-2-yl]carbamate (**11**) is synthesized by reacting 2-aminobenzimidazole (**10**) with methyl chloroformate in basic condition (Scheme 5).



Scheme 5

Methylthio analog of Albendazole is impurity F, methyl [5-(methylthio)-1*H*-benzimidazol-2-yl]carbamate (**15**) is synthesized in four steps from 2-nitroaniline (**2**). First step involves thiocyanation of 2-nitroaniline (**2**) yields 2-nitro-4-thiocyananiline (**3**) which was then reacted with methyl iodide to give corresponding methylthio derivative, 4-(methylthio)-2-nitroaniline (**12**). 4-(methylthio)-2-nitroaniline (**12**) was reduced using Fe/HCl to diamine compound, 4-(methylthio)benzene-1,2-diamine (**13**) which further reacted with methyl-*N*-cyanocarbamate sodium salt (**14**) to give methyl [5-(methylthio)-1*H*-benzimidazol-2-yl]carbamate (**15**) (Scheme 6). Methyl-*N*-cyanocarbamate sodium salt (**14**) can be obtained easily by reacting cyanamide with methyl chloroformate in presence of aqueous sodium hydroxide.



Scheme 6

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