



Synthesis, Characterization and Plant Growth Regulator Activity of Some Substituted 2-Amino Benzothiazole Derivatives

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ABSTRACT

Benzothiazole derivatives are the heterocyclic systems that have been found to exhibit diverse biological activities. Therefore, synthesis of benzothiazole derivatives as biologically active substances with various substituents in position 2 was undertaken. The title compounds were synthesized by facile synthetic procedure, starting with substituted aniline and potassium thiocyanate to give substituted 2-amino benzothiazoles, further addition of acid chlorides of substituted phenoxyacetic acid in basic condition was carried out to give the title compounds. All synthesized derivatives were characterized for structural confirmation by IR, ¹H NMR and Mass spectroscopy. The prepared compounds are screened for their plant growth regulator activity.

Keywords: Benzothiazole, phenoxyacetic acid, plant growth regulator.

INTRODUCTION

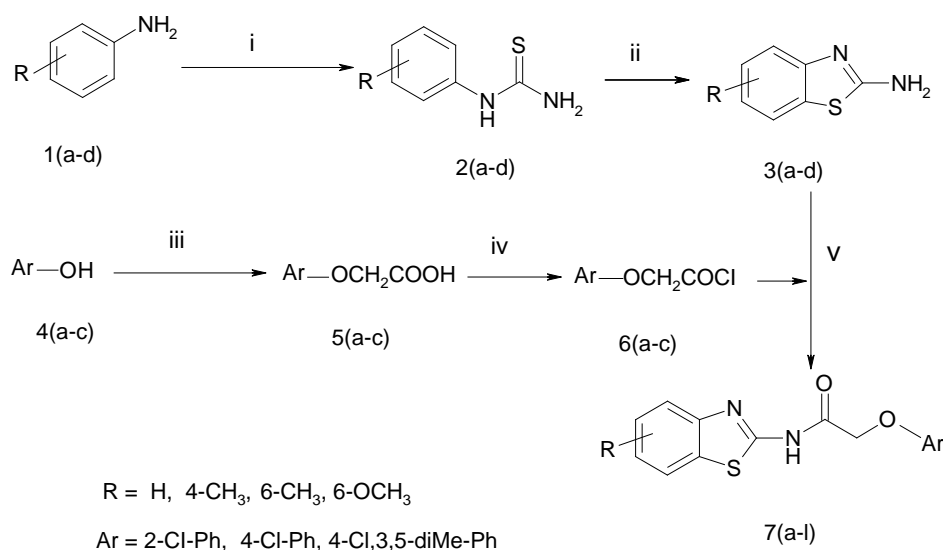
Benzothiazole and its derivatives represent a large group of heterocyclic compounds, some of which have already found application in the medical sphere as medicines (Kashiyama et al.) as well as in agriculture (Pulkrabek et al., Henselova et al.) as herbicides and plant growth regulators [1]. The benzothiazole derivatives act as herbicides [2], plant growth regulators [3, 4, 5, 6], antibacterial [7], anti-inflammatory [8], antifungal [9, 10], anticonvulsant [11], antitumor activity [12] and antiproliferative activity [13]. Nitrogen and sulfur containing ring is involved in several biologically active compounds. For example, Benzthiazuron and Methabenzthiazuron, derivatives of 2-aminobenzothiazole, were utilized as herbicides [2]. Substituted phenoxyacetic acids are biologically active compounds used as auxins in plant tissue culture. Commercially, they are also used as herbicides. Combination of substituted 2-aminobenzothiazole and substituted phenoxyacetic acid is an effective biologically active system that may be acting as proauxin. After application of these proauxin moieties they efficiently diffuse in the plant body, where they undergo metabolism at different rates and releases active parts. The seedling growth (i.e. emergence and elongation) depends on the environmental conditions and responses to phytohormone signaling pathways [14]. In view of this study, we aimed the synthesis of some substituted 2-amino benzothiazole derivatives as plant growth regulators.

MATERIALS AND METHODS

All the chemicals and solvents used in studies were of GR grade and were dried and purified before use. The purification of synthesized compounds was performed by recrystallisation with appropriate solvents. Purity of all the synthesized compounds was checked by TLC. Melting points of the synthesized compounds were determined by open capillary method and are uncorrected. IR spectra were recorded using Nujol with FT-IR Perkin-Elmer model Spectrum One Spectrophotometer, ^1H NMR spectra were recorded using CDCl_3 with Varian-300 spectrometer NMR instrument using TMS as an internal standard. Mass spectra were recorded in Agilent 6520Accurate-MassQ-TOF LC/MS.

Synthesis of phenyl thiourea 2(a-d): To aniline (0.1 mol) in water, concentrated HCl was added and the solution was warmed. A saturated solution of potassium thiocyanate (0.8 mol) in water was added slowly with stirring in above hydrochloride salt solution. Then the solution was heated for 4-5 h until the solution became turbid. The turbid solution was poured in cold water. The separated precipitate was filtered, washed with water and recrystallized from ethanol, so as to obtain pure compound phenylthiourea (Yield: 70-80%).

Synthesis of 2-aminobenzothiazole 3(a-d): A solution of Br_2 (0.12 mol) in carbon tetrachloride was added drop wise to a solution of phenylthiourea (0.1 mol) in carbon tetrachloride. The reaction mixture was stirred for 2.5 h at $15\text{-}20^\circ\text{C}$. Then solvent was evaporated and the residue was dissolved in hot water, filtered and neutralized by aqueous ammonia to obtain white precipitate, which was recrystallized from ethanol, so as to obtain pure 2-aminobenzothiazole (Yield: 97-99%).



Scheme 1: Reagents and conditions: (i) H_2O , HCl, KSCN, Heat; (ii) Br_2 , CCl_4 , $15\text{-}20^\circ\text{C}$; (iii) Chloroacetic acid, NaOH, Boil; (iv) SOCl_2 , CHCl_3 , Reflux; (v) Et_3N , CHCl_3 , $< 10^\circ\text{C}$.

Synthesis of phenoxyacetic acid 5(a-c): Substituted phenol (0.01 mol) and chloroacetic acid (0.012 mol) were transferred in a beaker and to it 6 ml, 7-8 N NaOH solution was added slowly with stirring. Reaction mixture was further diluted with 10 ml water and then concentrated to 50 %. The solution was cooled, diluted with water and neutralized with dilute HCl to get precipitate. This was dissolved in 10 % sodium bicarbonate solution, filtered through cotton plug and neutralized with dilute HCl to get pure product. Further recrystallisation from ethanol, resulted in pure substituted phenoxyacetic acid (Yield : >90%).

Synthesis of phenoxyacetyl chloride 6(a-c): A mixture of phenoxyacetic acid (0.01 mol) and thionyl chloride (0.02 mol) in 20 ml chloroform was refluxed for 1.5 h. The excess of thionyl chloride was removed by distillation. After cooling, a viscous liquid was obtained; this was immediately used for further reaction.

Synthesis of amide 7(a-l): A mixture of 2-aminobenzothiazole (0.01 mol) and triethylamine (0.02 mol) in 20 ml chloroform was stirred for 5 minutes. To it phenoxyacetyl chloride (0.01 mol) in chloroform was added drop wise. During addition the reaction temperature was maintained below 10 °C and the mixture was stirred for 4 h. The mixture was concentrated and small quantity of ethanol was added. The solid separated was washed with cold ethanol to get pure product.

2-(2-Chlorophenoxy)-N-(benzothiazol-2-yl) acetamide **7a**-Brown yellow solid, yield 70 %, m.p. 178-180°C IR cm⁻¹; 3362(N-H), 1746(C=O), 1597(C=N), 1456(C=C), ¹H NMR; (CDCl₃) δ: 4.91 (s, 2H, OCH₂-), 7.03-7.08 (t, 1H, Ar-H), 7.26-7.29 (d, 4H, Ar-H), 7.43-7.48 (t, 1H, Ar-H), 7.54-7.60 (t, 1H, Ar-H), 7.86-7.92 (t, 1H, Ar-H), >11 (s, 1H, NH), MS (ESI) *m/z*: 319 [M+H]⁺.

2-(2-Chlorophenoxy)-N-(4-methylbenzothiazol-2-yl) acetamide **7b**-Yellow solid, yield 72%, m.p. 182-184°C IR cm⁻¹; 3428(N-H), 1649(C=O), 1589(C=N), 1460(C=C), ¹H NMR; (CDCl₃) δ: 2.71(s, 3H, -CH₃), 4.80 (s, 2H, OCH₂-), 7.01-7.66 (m, 7H, Ar-H), >11 (s, 1H, NH).

2-(2-Chlorophenoxy)-N-(6-methylbenzothiazol-2-yl) acetamide **7c**-Brown solid, yield 68%, m.p. 196-198°C IR cm⁻¹; 3379(N-H), 1716(C=O), 1683(C=N), 1462(C=C), ¹H NMR; (CDCl₃) δ: 2.50 (s, 3H, -CH₃), 4.86 (s, 2H, OCH₂-), 7.01-7.07 (m, 2H, Ar-H), 7.24-7.34 (m, 2H, Ar-H), 7.43-7.46 (d, 1H, Ar-H), 7.65 (s, 1H, Ar-H), 7.74-7.76 (d, 1H, Ar-H), >11 (s, 1H, NH)

2-(2-Chlorophenoxy)-N-(6-methoxybenzothiazol-2-yl)acetamide **7d**-Brown solid, yield 69%, m.p. 190-192°C IR cm⁻¹; 3362(N-H), 1732(C=O), 1673(C=N), 1482(C=C), ¹H NMR; (CDCl₃) δ: 3.88 (s, 3H, -OCH₃), 4.88 (s, 2H, OCH₂-), 7.01-7.79 (m, 7H, Ar-H), >11 (s, 1H, NH)

2-(4-Chlorophenoxy)-N-(benzothiazol-2-yl) acetamide **7e**- Brown solid, yield 77%, m.p. 219-220°C, IR cm⁻¹; 3446(N-H), 1704(C=O), 1666(C=N), 1463(C=C), ¹H NMR; (CDCl₃) δ: 4.76 (s, 2H, OCH₂-), 6.97-7.00 (d, 2H, Ar-H), 7.29-7.34 (d, 2H, Ar-H), 7.39-7.44 (t, 1H, Ar-H), 7.50-7.55 (t, 1H, Ar-H), 7.83-7.88 (m, 2H, Ar-H), >11 (s, 1H, NH), MS (ESI) *m/z*: 319.05 [M+H]⁺.

2-(4-Chlorophenoxy)-N-(4-methylbenzothiazol-2-yl) acetamide **7f** - Brown yellow solid, yield 70%, m.p. 215-217°C, IR cm⁻¹; 3379(N-H), 1721(C=O), 1571(C=N), 1463(C=C), ¹H NMR; (CDCl₃) δ: 2.78 (s, 3H, -CH₃), 4.84 (s, 2H, OCH₂-), 7.04-7.74 (m, 7H, Ar-H), 13.40 (s, 1H, NH).

2-(4-Chlorophenoxy)-N-(6-methylbenzothiazol-2-yl) acetamide **7g**- Brown yellow solid, yield 82%, m.p. 188-190°C, IR cm⁻¹; 3374(N-H), 1688(C=O), 1605(C=N), 1464(C=C), ¹H NMR; (CDCl₃) δ: 2.49 (s, 3H, -CH₃), 4.72 (s, 2H, OCH₂-), 6.92-7.69 (m, 7H, Ar-H), >11(s, 1H, NH).

2-(4-Chlorophenoxy)-N-(6-methoxybenzothiazol-2-yl) acetamide **7h** - Light yellow solid, yield 61%, m.p. 198-200°C, IR cm⁻¹; 3376(N-H), 1686(C=O), 1654(C=N), 1466(C=C), ¹H NMR; (CDCl₃) δ: 3.88 (s, 3H, -OCH₃), 4.72 (s, 2H, OCH₂-), 6.91-6.93 (d, 2H, Ar-H), 7.05-7.09 (d, 1H, Ar-H), 7.31 (s, 1H, Ar-H), 7.31-7.34 (d, 2H, Ar-H), 7.69-7.72 (d, 1H, Ar-H), 9.72 (s, 1H, NH).

2-(4-Chloro-3,5-dimethylphenoxy)-N-(benzothiazol-2-yl) acetamide **7i** - Light yellow solid, yield 82%, m.p. 196-198°C, IR cm⁻¹; 3396(N-H), 1697(C=O), 1642(C=N), 1455(C=C), ¹H NMR; (CDCl₃) δ: 2.36 (s, 6H, 2-CH₃), 4.72 (s, 2H, OCH₂-), 6.68 (s, 1H, Ar-H), 6.77 (s, 1H, Ar-H), 7.26-7.86 (m, 4H, Ar-H), 11.92 (s, 1H, NH).

2-(4-Chloro-3,5-dimethylphenoxy)-N-(4-methylbenzothiazol-2-yl) acetamide **7j** - Yellow solid, yield 74%, m.p. 230-232^oC, IR cm⁻¹; 3398(N-H), 1703(C=O), 1589(C=N), 1464(C=C), ¹H NMR; (CDCl₃) δ: 2.37 (s, 6H, 2-CH₃), 2.65 (s, 3H, -CH₃), 4.70 (s, 2H, OCH₂-), 6.74 (s, 2H, Ar-H), 7.21-7.30 (m, 2H, Ar-H), 7.66-7.68 (m, 1H, Ar-H), 9.83 (s, 1H, NH)

2-(4-Chloro-3,5-dimethylphenoxy)-N-(6-methylbenzothiazol-2-yl) acetamide **7k** - Brown yellow solid, yield 76%, m.p. 202-204^oC, IR cm⁻¹; 3397(N-H), 1714(C=O), 1590(C=N), 1465(C=C), ¹H NMR; (CDCl₃) δ: 2.36 (s, 6H, 2-CH₃), 2.49 (s, 3H, -CH₃), 4.71 (s, 2H, OCH₂-), 6.77 (s, 2H, Ar-H), 7.26 (s, 1H, Ar-H), 7.64-7.69 (m, 2H, Ar-H), >11 (s, 1H, NH).

2-(4-Chloro-3,5-dimethylphenoxy)-N-(6-methoxybenzothiazol-2-yl) acetamide **7l** - Brown solid, yield 63%, m.p. 154-156^oC, IR cm⁻¹; 3394(N-H), 1710(C=O), 1605(C=N), 1472(C=C), ¹H NMR; (CDCl₃) δ: 2.36 (s, 6H, 2-CH₃), 3.88 (s, 3H, -OCH₃), 4.69 (s, 2H, OCH₂-), 6.70 (s, 2H, Ar-H), 7.26 (s, 1H, Ar-H), 7.04-7.07 (d, 1H, Ar-H), 7.29 (s, 1H, Ar-H), 7.67-7.70 (d, 1H, Ar-H), 9.74 (s, 1H, NH)

RESULTS AND DISCUSSION

Chemistry: In the present study, the preparation of twelve acetamide derivatives of substituted 2-aminobenzothiazole using phenoxyacetic acid is described. Evidence for their structures by means of IR, ¹H NMR and Mass spectrum completed the knowledge about acylation of the 2-aminobenzothiazoles using triethylamine as a base in chloroform. In case of the intermediate i.e. thiourea derivative, the reaction is very sensitive and it is necessary to use sufficient quantity of concentrated HCl (not too excess) and also it is necessary to keep heating below the reflux or boiling temperature, otherwise, the product obtained is undesirable. In case of phenoxyacetic acids 5a and 5b, the purification by using 10% NaHCO₃ solution was possible but not possible for 5c, due to bulkiness of the phenoxy ring.

Spectrum : In general, the IR spectra of the synthesized acetamide derivatives show the absorption bands at 3362-3446 cm⁻¹ for N-H group, The absorption bands of the C=O group were observed in the range 1649-1746 cm⁻¹. The IR absorptions for the C=N group were observed at 1571-1683 cm⁻¹ and that for C=C group in 1455-1482 cm⁻¹. As evident from the spectrum of the individual derivative, these bands are dependent on the substituent.

In the ¹H NMR spectra the signals of protons of the OCH₂-CO group appeared as singlets in the region of 4.69-4.91 ppm. The signals of aromatic protons appeared as multiplets in the range 7.01-7.86 ppm. The signals for NH protons were observed at 9.71-13.40 ppm as singlets and were D₂O- exchangeable. In case of compounds **7b**, **7f** and **7j** the -CH₃ (substituted on 4-position of benzothiazole ring) protons signals were observed at 2.71, 2.78 and 2.65 ppm respectively. While, in compounds **7c**, **7g** and **7k** the -CH₃ (substituted on 6-position of benzothiazole ring) protons signals were observed at 2.50, 2.49 and 2.49 ppm respectively. In case of compound **7d**, **7h** and **7l** the -OCH₃ protons signals were observed at the same position 3.88 ppm. For compounds **7i**, **7k** and **7l** the signals of 2-CH₃ protons of phenoxy group appeared at 2.36 ppm, while, that for **7j** at 2.37 ppm.

APPLICATIONS

Plant Growth Regulator activity: The plant growth regulator activity of synthesized compounds was investigated against wheat seeds. All compounds were tested for their effects on the growth of germinating seeds by a technique similar to that of FOLKE SKOOG [15], in this modification: Seeds of wheat were germinated on filter paper in Petri dishes. Ten seeds were used in each Petri dish, moistened with 10 mL of a water solution of the compound to be tested. Usually serial concentrations of 1, 2.5, 5, 50 and 100 ppm were used and all treatments were in duplicate. The Petri-dishes were placed in a dark room with approximately constant temperature (25 ±2 ^oC) four days. After four days of growth in a dark, the

seedlings were removed and length of the shoots & primary roots were measured (tables 1, 2 and figures 1, 2). The growth effect of tested compounds were evaluated and compared with the control and standards in the same range of concentrations.

Table1: Average shoot length of wheat seedlings.

Compounds	Shoot length (mm)				
	100 ppm	50 ppm	5 ppm	2.5 ppm	1 ppm
7a	08.55	26.37	50.75	57.40	52.00
7b	16.00	24.66	45.22	38.77	47.33
7c	17.33	31.40	53.87	69.44	62.20
7d	16.25	38.33	57.66	56.80	56.40
7e	09.00	30.16	46.14	51.60	62.77
7f	08.50	15.62	18.20	30.22	48.60
7g	08.20	16.83	25.11	49.66	38.20
7h	13.50	16.44	24.22	16.20	93.60
7i	15.75	19.66	43.55	45.22	51.33
7j	16.00	20.22	45.70	41.30	52.20
7k	21.50	29.80	65.62	52.30	52.20
7l	10.50	29.50	43.60	35.00	42.00
4-CPA	00	04.66	15.30	18.11	22.44
2,4-D	00	13.00	13.37	14.00	13.90
Control	39.40				

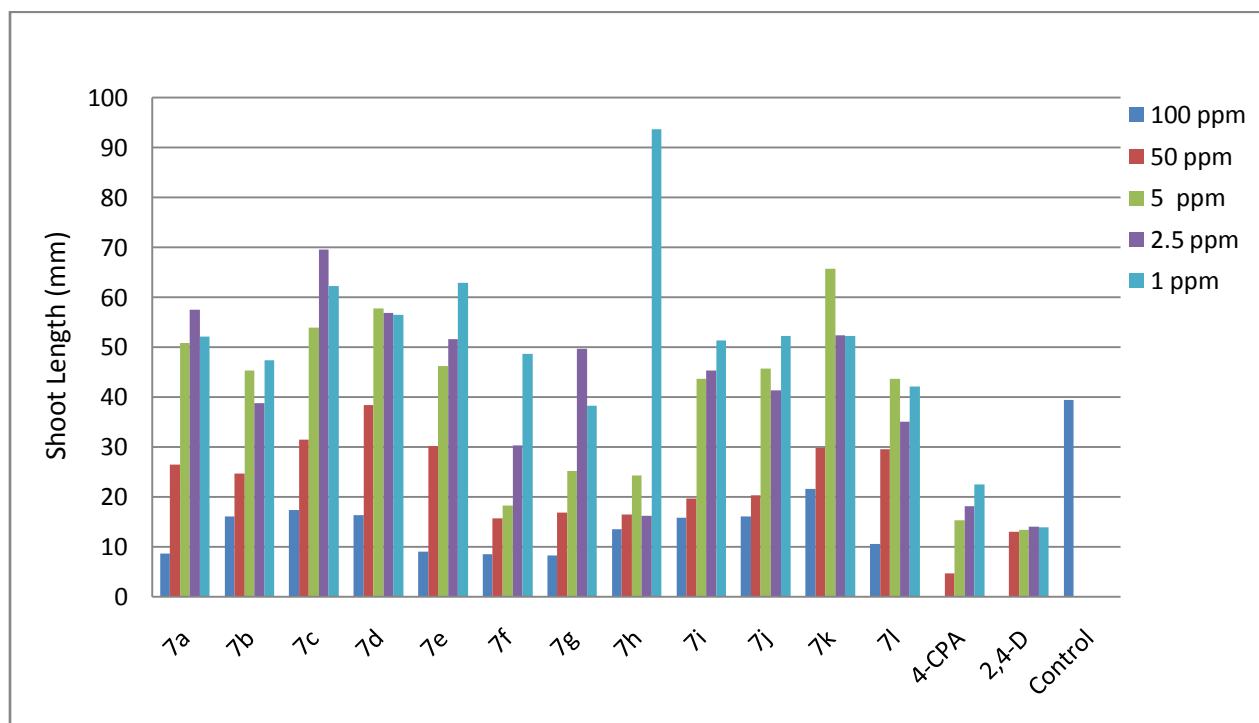
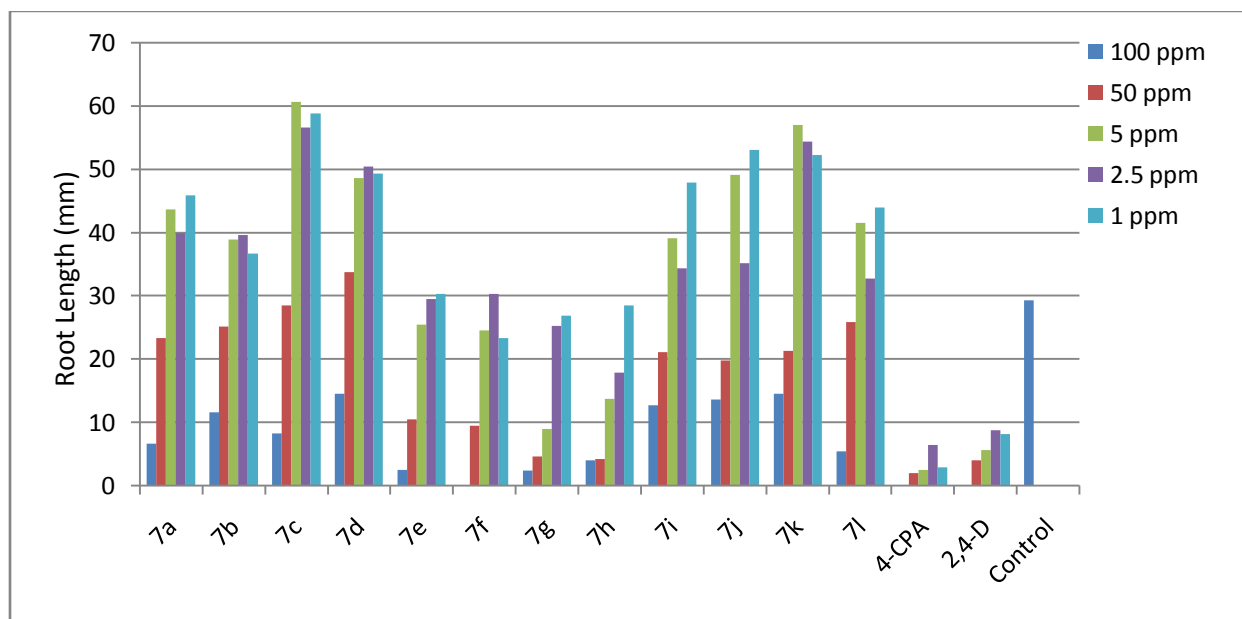


Figure1: Average shoot length of wheat seedlings.

Table 2: Average root length of wheat seedlings.

Compounds	Root length (mm)				
	100 ppm	50 ppm	5 ppm	2.5 ppm	1 ppm
7a	06.66	23.37	43.62	39.9	45.90
7b	11.60	25.11	38.88	39.55	36.66
7c	08.33	28.50	60.62	56.55	58.80
7d	14.50	33.77	48.55	50.40	49.30
7e	02.50	10.50	25.42	29.50	30.33
7f	00	09.50	24.50	30.33	23.30
7g	02.40	04.60	09.00	25.22	26.90
7h	04.00	04.22	13.77	17.90	28.50
7i	12.75	21.11	39.11	34.33	47.88
7j	13.66	19.77	49.10	35.20	53.00
7k	14.50	21.30	57.00	54.30	52.20
7l	05.50	25.87	41.50	32.70	43.90
4-CPA	00	02.00	02.50	06.44	02.88
2,4-D	00	04.00	05.62	08.80	08.20
Control	29.30				

**Figure 2:** Average root length of wheat seedlings.

CONCLUSIONS

The effects of the synthesized acetamide derivatives **7a-l** were tested on the germination of wheat seeds. Almost, all the compounds showed good activity at tested concentrations. Low concentrations of acetamide derivatives 1-5 ppm stimulated the growth of wheat seedlings (shoot/hypocotyls and roots) but concentrations in the range of 50-100 ppm found to be inhibitory. The compounds **7a**, **7c**, **7d**, **7e**, **7h** & **7k** showed growth stimulating activity for shoots. While, the most effective compounds with stimulating activity for root growth were **7a**, **7b**, **7c**, **7d**, **7i**, **7j**, **7k** & **7l**. Among all the compounds **7h** at 1 ppm

concentration displayed excellent shoot growth. The tested derivatives may be characterized as biologically active substances with auxin like growth promoting activity.

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