**Development of Crown ethers containing neonicotinoid derivative: synthesis, characterization, biological activities**

**M. B. Deshmukh1, S. M. Deshmukh1 , S. R. Yankanchi1 and Bhagawan Patil1\***

1Dept of Agrochemical & Pest Management, Shivaji-University, Vidyanagar, Kolhapur-Maharashtra, INDIA.

\* Email id: bvpatil777@gmail.com

**ABSTRACT**

The structures of the newly synthesized compounds were confirmed by FT-IR, 1H NMR, 13C NMR, and Mass spectroscopic data. The entire prepared molecule is screened to investigate its Insecticidal & anti-bacterial activity. The bioassay tests showed that all synthesized compounds showed higher bioactivities than imidacloprid against *H. armigera* (Hub), Mealybugs, Mango hoppers, and Tobacco & Tomato bacterial wilt. The results of biological activity were analyzed statistically. Crown analog-containing compounds demonstrated potential as vector control agents, advancing agricultural research for increased production of crops including tobacco and tomatoes.

**Keywords:** Substituted crown, Synthesis, Spectral Characterization, Biological Activity.

**INTRODUCTION**

The insect pests *H. armigera* (Hübner), mealybugs (*Planococcus citri*), and mango hoppers (*Idioscopus clypealis*) are known to be damaging to many commercially significant crops worldwide, including cotton, corn, tobacco, soybeans, and tomatoes1. Nicotine terminates the insects quickly within an hour causing tremors, convulsions, and subsequently paralysis. Before 1746, the insecticidal activities of crude extract of tobacco leaves were used to control the insects. Metcalf has reported that 1.2 million pounds of free nicotine were used in Agriculture in the USA during 19442. Some biological characteristics, such as mobility and facultative diapauses, can increase the survival and population upsurge of the pest in agricultural systems 3. This pestilence, which attacks more than 150 different host species, is considered the most commercially important insect pest in many countries, such as Japan, China, India, and Southeast Asia4. Due to their biological characteristics and more damage potential, successful prevention and control of these pests become tough. The prevention and control of H. armigera are mainly dependent on chemical pesticides5. However, total dependence on the application of synthetic insecticides to control H. armigera has not achieved the desired success and has resulted in the unfolding of pesticide resistance, environmental contamination, disruption of ecological stability, and health hazards6. Neonicotinoid insecticides are the latest class of synthetic insecticides in the past two-decade and the biggest selling insecticide class worldwide through compounds such as imidacloprid7. Thus, numerous attempts have been made to find replacement methods for its control. Recently, a crown moiety8 was found to be very notable in the discovery of novel insecticides and several modifications around its structure have been reported. New insecticidal molecules are developed in the present work based on the following: incorporation of the crown analogue of imidacloprid. Based on this hypothesis, a crown containing imidacloprid derivative is designed and synthesized (scheme). Biological assays reveal that the synthesized compound exhibits excellent insecticidal and antibacterial activities against different insect species.

**EXPERIMENTAL**

Material & Methods: The chemicals used in the present studies were of synthetic grade, and some were sourced from Merck Company Ltd. The products were characterized by IR (FT/IR 4100 Spectrophotometer using KBr disc), 1H NMR (Bruker 1H NMR 400MHZ) using TMS as an internal standard & Mass spectra obtained by QP2010 (Shimadzu) spectrometer. Melting point determination was taken in open capillary tubes and is uncorrected. Thin layer chromatography is performed with E. Merck pre-coated silica gel plates with iodine as a spot-developing chemical agent.

**Compound 1: Synthesis of 2-chloro-5-{[-2-hydrazinylideneimidazolidin-1-yl] methyl} pyridine**

1-[(6-chloropyridin-3-yl) methyl]-N-nitroimidazolidin-2-imine {Imidacloprid (1 mmol)} and SnCL2.2H20 (0.5mmol) in 10 ml of absolute ethanol further heated on a steam bath 70°C -80°C; the completion of the reaction the mixture is allowed to cool & poured into ice water mixture. The pH of the solution was made alkaline by 5% NaOH & then extracted with ethyl acetate. The organic phase is thoroughly washed with Braine solution and dried over sodium sulphate to get a yellowish brown-colored compound, yield 79%.

**2-chloro-5-{[-2-hydrazinylideneimidazolidin-1-yl]methyl}pyridine** (1) Yield (79%), m.p. 148°C; IR (KBr, ν cm1 ) : 3408, 3302(NH str ), 2908(CH2 str ), 1617(C=N str ), 1444(CH=CH str), 758(C-Cl str ), 1H NMR (CDCl3, ppm ) δ 2.06 (s, 2H, CH2), 3.51 ( t, J=7.5 HZ, CH2), 3.62 ( t, J=7.5 HZ,CH2), 7.72 (d, J=7.5 HZ,Py1H) , 8.30 (s,Py1H), 8.99 (s, NH) ; 13C NMR (CDCl3, ppm ) 159, 151, 150, 137, 133, 124, 51, 50, 46 ; MS (C15H13N5) , (m/z) : 225, 189, 183, 165, 125, 100, 99, 87, 84, 69 (M+ )

**Compound 2: Synthesis of 4 –formyl mono-benzo 18-crown-6**

A mixture of mono-benzo 18-crown-6 (5.6 mmol), TFA (8.3 ml), and hexamethylenetetramine (3.22gm,22 mmol) was stirred at 90°C under nitrogen for 24 hrs. The mixture was extracted with benzene, and the extract was dried (MgSO4). The concentration of the benzene extract under vacuum gave yellow oil, which solidified to brown crystals upon cooling. Finally, the obtained products were dried and recrystallized with ethanol.

**4 –formyl mono-benzo 18-crown-6** (2) Yield (77%), m.p. 233°C; IR (KBr, ν cm1 ): 2921, 2850 (CH2 str ), 1646(C=O str),1568,1356(C=C str ), 1257,1131,1120(C-O-C str), 989(Ar-CH str ), 1H NMR (CDCl3, ppm ) δ 3.64-3.65 ( t, J=7.5 HZ, CH2), 3.72-3.83 ( t, J=7.5 HZ, CH2), 4.28-4.29 ( t, J=7.5 HZ, CH2), 6.92-7.44 (m, Ar-CH), 9.82 (s, CHO); 13C NMR (CDCl3, ppm ) 191, 154, 147, 130, 125, 110, 109, 71, 70, 69; MS (C17H24O7), (m/z) 340, 312, 286, 236, 235, 209, 165, 151, 119, 117,75,71 (M+ )

**Compound 3 : Synthesis of 1-(1-((6-chloropyridin-3-yl)methyl)imidazolium-2- ylidene) Monobenzo-18-crown-6**

4 –formyl mono-benzo 18-crown-6 (0.25 mmol) and Imidacloprid Aminoguanidine (0.25 mmol) were dissolved in DMF (2ml), then p-toluene sulphonic acid (0.1 mmol) was added. The mixture was stirred at 80°C for 6-7 hrs. The reaction mixture was cooled to RT and then added dropwise with vigorous stirring into a mixture of Na2CO3 (0.1 mmol) and water (20 ml). Finally, the obtained products were dried and recrystallized with ethanol.

**(E)-1-(1-((6-chloropyridin-3-yl)methyl)imidazolidin-2- ylidene) Monobenzo-18-crown-6** (3) Yield (63%), m.p. 152°C; IR (KBr, ν cm1 ) : 3302(NH str ), 2921,2850 (CH2 str ), 1646(C=O str),1618(C=N str ), 1568,1444(CH=CH str), 1257,1131,1120(C-O-C str), 758(C-Cl str ), 1H NMR (CDCl3, ppm ) δ 2.06 (s, 2H, CH2),2.68-2.77(t),2.98-3.00(t),3.09-3.14(t),3,49, 3.51 ( t, J=7.5 HZ, CH2), 3.61 ( t, J=7.5 HZ, CH2), 3.56,3.60-3.64(t), 3.73,3.22-4.20(t), 4.03, 6.84, 7.14, 7.35, 7.92-7.94, 8.30 (s,Py1H) ; 13C NMR (CDCl3, ppm ) 152, 151, 150, 148, 145, 138, 134, 127, 123, 122, 113, 111, 93, 72, 71, 70, 63, 46 ,41 ; MS (C15H13N5) , (m/z) : 549, 523, 521, 488, 524, 354, 339, 286, 236, 212, 210, 125, 87 (M+ ).

**Compound 4: synthesis of 4,4’ –diformyl dibenzo 18-crown-6**

A mixture of Dibenzo 18-crown-6 (5.6 mmol), TFA (8.3 ml), and hexamethylene tetramine (3.22gm,22 mmol) was stirred at 90°C under nitrogen for 24 hrs. The mixture was extracted with benzene, and the extract was dried (MgSO4). The concentration of the benzene extract under vacuum gave yellow oil, which solidified to brown crystals upon cooling. Finally, the obtained products were dried and recrystallized with ethanol.

**4,4’ –diformyl monobenzo 18-crown-6** (4) Yield (72%), m.p. 234°C; IR (KBr, ν cm1 ): 2921,2850 (CH2 str ), 1647(C=O str ), 1568, 1356,1257(CH=CH str),1131,1120(C-O-C), 989(Ar-CH), 1H NMR (CDCl3, ppm ) δ 3.52 ( t, J=7.5 HZ, CH2), 3.69 ( t, J=7.5 HZ, CH2), 4.16-4.24(t) 4.58.4.73,7.37-7.44(m, ArCH), 9.82 (s, CHO), 13C NMR (CDCl3, ppm ) 191, 150, 148, 131, 128, 111, 110,71,70, 69; MS (C17H24O8), (m/z): 416, 390,388,362,312,80 (M+ )

**Compound 5 : Synthesis of (E)-1,3-(1-((6-chloropyridin-3-yl)methyl)imidazolium-2- ylidene) dibenzo-18-crown-6**

4,4’ –diformyl dibenzo 18-crown-6 (0.25 mmol) and Imidacloprid Aminoguanidine (0.50 mmol) were dissolved in DMF (2ml), then p-toluene sulphonic acid (0.1 mmol) was added. The mixture was stirred at 80°C for 6-7 hrs. The reaction mixture was cooled to RT and then added dropwise with vigorous stirring into a mixture of Na2CO3 (0.1 mmol) and water (20 ml). The product was filtered and recrystallized from ethanol.

**(E)-1,3-(1-((6-chloropyridin-3-yl)methyl) imidazolium-2- ylidene) dibenzo-18-crown-6** (5) Yield (81%), m.p. 156°C; IR (KBr, ν cm1 ): 3302(NH str ), 2921,2850(CH2 str ), 1618(C=N str ), 1568,1444(CH=CH str), 1131,1120(C-O-C), 758(C-Cl str ), 1H NMR (CDCl3, ppm ) δ 2.60 (s, 2H, CH2), 2.98.3.52 ( t, J=7.5 HZ, CH2), 3.69 ( t, J=7.5 HZ, CH2),3.94-,4.16. 4.24(t).4.58. 4.73. 6.80-7.93 7.72 (d, J=7.5 HZ, Py1H), 8.52, 8.30 (s, Py1H), 13C NMR (CDCl3, ppm) 152, 151, 150, 144, 138, 137, 134, 127, 123, 121, 112, 111, 94, 71, 62, 50,4 6,41; MS (C15H13N5), (m/z) 834, 808. 806, 773, 709, 665, 639, 624, 622, 521, 289, 212. 210, 195, 169, 125, 87. (M+).





**INSECTICIDAL ACTIVITY 9, 10 :**

To make 300, 600, and 800 mg litre-1 concentrations of the standard solutions of the standard and synthetic compounds, they were dissolved in 1% acetone, 1% DMF, and 0.1% Tween-20 solution. By soaking fresh tobacco leaves in various concentration solutions and feeding them to Mealybugs, these chemicals were treated orally. Similar to this, treated fresh inflorescence was fed to Mango Hopper Nymph and Hub. The mortality data were collected, after 72 hrs. Of treatment and presented in **Table-1**.

**Table-1:** Mortality data of treated compounds against sucking insect pests.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Compound Name | Concentrations  mg. liter-1 | Mortality after 24 hrs. of treatment | | |
| H.armigera(Hub)\* | Mealybugs\* | Mango hoppers\* |
| 1 | 300 | 62 | 58 | 91 |
| 600 | 98 | 88 | 96 |
| 800 | 100 | 100 | 100 |
| 3 | 300 | 66 | 68 | 92 |
| 600 | 98 | 89 | 97 |
| 800 | 100 | 100 | 100 |
| 5 | 300 | 66 | 68 | 95 |
| 600 | 98 | 90 | 96 |
| 800 | 100 | 100 | 100 |
| Imidacloprid | 300 | 52 | 46 | 90 |
| 600 | 100 | 100 | 100 |
| 800 | 100 | 100 | 100 |
| Control (Solvent) | -- | 5 | 4 | 8 |

**Table-2:** Mortality data of treated compounds against sucking insect pests.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Compound Name | Concentrations  mg. liter-1 | Mortality after 48 hrs. of treatment | | |
| H.armigera(Hub)\* | Mealybugs\* | Mango hoppers\* |
| 1 | 300 | 62 | 58 | 91 |
| 600 | 98 | 88 | 96 |
| 800 | 100 | 100 | 100 |
| 3 | 300 | 62 | 58 | 91 |
| 600 | 100 | 100 | 100 |
| 800 | 100 | 100 | 100 |
| 5 | 300 | 62 | 58 | 91 |
| 600 | 100 | 100 | 100 |
| 800 | 100 | 100 | 100 |
| Imidacloprid | 300 | 52 | 46 | 90 |
| 600 | 100 | 100 | 100 |
| 800 | 100 | 100 | 100 |
| Control (Solvent) | -- | 5 | 4 | 8 |

**Table-3:** Mortality data of treated compounds against sucking insect pests.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Compound Name | Concentrations  mg. liter-1 | Mortality after 24 hrs. of treatment | | |
| H.armigera(Hub)\* | Mealybugs\* | Mango hoppers\* |
| 1 | 300 | 62 | 58 | 91 |
| 600 | 98 | 88 | 96 |
| 800 | 100 | 100 | 100 |
| 3 | 300 | 62 | 58 | 91 |
| 600 | 100 | 100 | 100 |
| 800 | 100 | 100 | 100 |
| 5 | 300 | 62 | 58 | 91 |
| 600 | 100 | 100 | 100 |
| 800 | 100 | 100 | 100 |
| Imidacloprid | 300 | 59 | 54 | 90 |
| 600 | 100 | 100 | 100 |
| 800 | 100 | 100 | 100 |
| Control (Solvent) | -- | 5 | 4 | 8 |

\*Means of six replications

**ANTIBACTERIAL ASSAY**

A turbidimeter test11 was used to assess each compound's antibacterial activity against tobacco bacterial wilt and tomato bacterial wilt 10. Kocide ® 3000 (Cu (OH)) was employed as the positive control(200mg/L). The substances were dissolved in 150 L of DMSO, diluted to final concentrations of 300, 600, and 800 mg/L with water containing Tween-20 (0.1%, Tween-20: water, v/v), and then introduced to nutritional broth (NB) liquid medium in 5 mL tubes. Each of these tubes received around 40 L of NB liquid media containing the solanacearum pathogen. 48 hours of shaking at 30°C and 180 rpm came next. The following equation was used to compute the relative inhibition rate of the circular mycelium in comparison to the blank test.

Relative inhibitory rate (%) = [(A0 – A1 )/A0 ] × 100%

A0: Corrected OD values of the control medium of bacilli.

A1: Corrected OD values of the medium of toxicity.

**Table-4: The antibacterial activity of synthesized compounds & Imidacloprid against Tobacco bacterial wilt and Tomato bacterial wilt at 300, 600, and 800 mg/L.**

|  |  |  |  |
| --- | --- | --- | --- |
| Compound Name | Concentrations  mg. liter-1 | **Tobacco bacterial wilt (%)** \* | **Tomato bacterial wilt (%)** \* |
|
| 1 | 300 | 44 | 52 |
| 600 | 58 | 74 |
| 800 | 72 | 84 |
| 3 | 300 | 45 | 53 |
| 600 | 59 | 76 |
| 800 | 82 | 88 |
| 5 | 300 | 47 | 59 |
| 600 | 61 | 79 |
| 800 | 83 | 90 |
| Imidacloprid | 300 | 42 | 40 |
| 600 | 54 | 56 |
| 800 | 69 | 66 |
| Kocide® 3000 (Cu(OH) ) | 200 | 100 | 100 |

\*Means of six replications

**RESULTS AND DISCUSSION**

Chemistry: According to the plan, imidacloprid was reduced to yield 2-chloro-5-[-2-hydrazinylideneimidazolidin-1-yl] methyl pyridine (2), which was then produced by raising the pH of the solution to an alkaline level using 5% sodium hydroxide before being extracted with ethyl acetate. To get the yellowish brown color product (79% yield), the organic phase is completely washed with Braine solution and dried on sodium sulphate. Compound 1's IR spectra revealed the distinctively strong absorption bands of NH and NH2 at 3302, 3408, and 1617 cm-1, respectively (Scheme). Proton NH2 1H-NMR singlet signals show at 8.99 and 2.06 (s, 2H, CH2), 3.51 (t, J=7.5 HZ, CH2), 3.62 (t, J=7.5 HZ, CH2), 7.72 (d, J=7.5 HZ, Py1H), and 8.30 (s, Py1H) ppm with Carbon NMR spectrums 159,151,150,137,133,124,51,50,46, respectively. The final compounds, sl. nos. 3 and 5, likewise showed in their IR spectra prominent NH absorption bands at 3302 cm-1 and 1617 cm-1, respectively (Scheme), and the aldehydic peak was eliminated at 1650. Compound 4 displays the 1H-NMR spectrum and the protons' CHO singlet signals vanish and shows δ 2.06 (s, 2H, CH2),2.68-2.77(t),2.98-3.00(t),3.09-3.14(t),3,49, 3.51 ( t, J=7.5 HZ, CH2), 3.61 ( t, J=7.5 HZ, CH2), 3.56,3.60-3.64(t), 3.73,3.22-4.20(t), 4.03, 6.84, 7.14, 7.35, 7.92-7.94, 8.30 (s, Py1H) ppm with Carbon NMR spectrums 152, 151, 150, 148, 145, 138, 134, 127, 123, 122, 113, 111, 93, 72, 71, 70 respectively. Compound 5 also displays The 1H-NMR spectrum singlet signals of protons CHO disappear and shows δ 2.60 (s, 2H, CH2), 2.98.3.52 ( t, J=7.5 HZ, CH2), 3.69 ( t, J=7.5 HZ, CH2),3.94-,4.16. 4.24(t).4.58. 4.73. 6.80-7.93 7.72 (d, J=7.5 HZ, Py1H), 8.52, 8.30 (s, Py1H) ppm with Carbon NMR spectrums 152, 151, 150, 144, 138, 137, 134, 127, 123, 121, 112, 111, 94, 71 respectively. Compounds 3 and 5 displayed Mass spectrum the characteristic sharp peak of compound 3 are 549, 523, 521, 488, 524, 354, 339, 286, 236, 212, 210, 125, 87 (M+), and compound 5 are 834, 808. 806, 773, 709, 665, 639, 624, 622, 521, 289, 212. 210, 195, 169, 125, 87. Together with other spectral data, the proton and carbon NMR spectral data, as well as the mass spectral fragmentation pattern, validated the structure of the produced compounds.

**Biological Activity**: **Insecticidal Activity,** the death rates caused by synthesized new neonicotinoid derivatives for H. armigera (Hub), Mealybugs (Planococcus citri), and Mango Hoppers (Idioscopus clypealis) are displayed in Table 1 below. All insects died at a greater rate when exposed to the 800 mg litre-1 concentration solution than when exposed to any other synthetic compound concentration. The majority of the synthetic compounds demonstrate good insecticidal activity against various insect species, according to biological experiments.

**Antibacterial Activity:** The synthesized molecule was assessed for its antibacterial efficacy against tobacco bacterial wilt and tomato bacterial wilt was examined by a Turbidimeter test. The antibacterial activity of synthesized substance and imidacloprid was evaluated using the Kocide® 3000 standard.

**CONCLUSION**

Several linear crowns containing imidacloprid were synthesized in the current work by these therapeutic properties. Compound 1 is a new neonicotinoid derivative made from 1-[(6-chloropyridin-3-yl) methyl]-N-nitroimidazolidin-2-imine and 2-chloro-5-[-2-hydrazinylideneimidazolidin-1-yl] methyl pyridine (Imidacloprid). Compounds 3 and 4 are the ultimate crown analogs of imidacloprid, and they are created by treating them with 2-chloro-5-[-2-hydrazinylideneimidazolidin-1-yl] methyl pyridine and 4,4’-formyl dibenzo-18-crown-6, respectively. All of the recently synthesized compounds were evaluated for their insecticidal and antibacterial capabilities, and they were all described using a variety of contemporary analytical methods. According to preliminary biological activity testing, the title chemical has more effective insecticidal properties against Mealybugs. Similar results were obtained with mango hopper nymphs and H. armigera (Hub) at 300, 600, and 800 mg/L, respectively. Also, at a concentration of 800 mg/L, synthetic drugs, and imidacloprid showed superior antibacterial activity against Pseudomonas solanacearum (including tobacco bacterial wilt and tomato bacterial wilt). The acquired results are encouraging, which validated that this work is valuable for further study on the creation of novel, efficient bactericides, and pesticides that may aid with being utilized in management strategies for vector control.

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