IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 11, Issue 6 Ver. I (June. 2018), PP 63-70
www.iosrjournals.org

Synthesis of Allosteric Modulators of CB1 Cannabinoid Receptors

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Abstract: *Sample compounds that could act as allosteric modulators of CB1 cannabinoid receptors were synthesized. The compounds are analogues of pre-discovered bioactive molecules that bind to the CB1 allosteric site. Allosteric modulation could be a new effective approach to manipulating the endocannabinoid system by enhancing the signaling of already existing selective agonists, inverse agonists and enzyme inhibitors.Fischer indole synthesis, Vilsemeier-Haack reaction, $InBr_3$-catalyzed sulphonation of indoles, reductive amination of indoles, bromination of indoles, were the synthesis reactions applied. Seven analogues were obtained. Percentage yield and melting point determination were performed for each compound. ^1H and ^{13}C NMR spectroscopy were also applied for characterization.*

Keywords: *allosteric modulators, cannabinoid receptors, endocannabinoid system, bioactive molecules, agonists, enzyme inhibitors.*

Date of Submission: 12-06-2018 Date of acceptance: 30-06-2018

I. Introduction

Cannabis sativa, an annual herb in the *cannabacae* family has many common names such as hemp, marijuana, bhang, ganja, hashish. This herb has been used throughout recorded history as a source of fiber, for its seed oil, as food, as a drug, as medicine and for spiritual purposes. Cannabis is one of the first plants to have been used as a medicine, for religious ceremonies and recreationally, the first account of its use for these purposes stretching back 5000 years ago. However the findings that cannabis is a unique source of a set of at least 66 compounds known as cannabinoids and that the psychotropic effects of cannabis are produced mainly by (⁻)-trans-Δ^9-tetrahydrocannabinol (Fig. 1) are much more recent. The tetrahydrocannabinols have documented medicinal value.

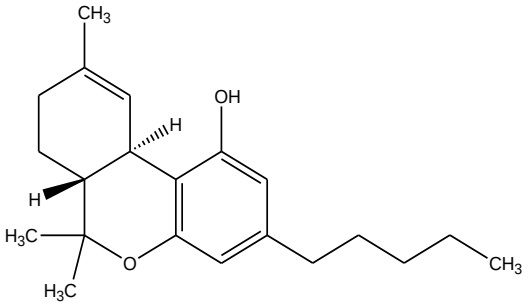


Fig. 1: Δ^9-tetrahydrocannabinol

Early indications of the existence of cannabinoid receptors came from reports firstly, that the pharmacological activity of psychotropic cannabinoids is significantly influenced by chemical structure, secondly, that cannabinoids with chiral centres exhibit stereoselectivity, and thirdly, that the potency of Δ^9-THC matches that of agonists for at least some established classes of receptors.

Cannabinoid receptors can be engaged directly by agonists or antagonists or indirectly by manipulating endocannabinoid metabolism. They are activated by three major group of ligands, endocannabinoids, plant cannabinoids and synthetic cannabinoids.

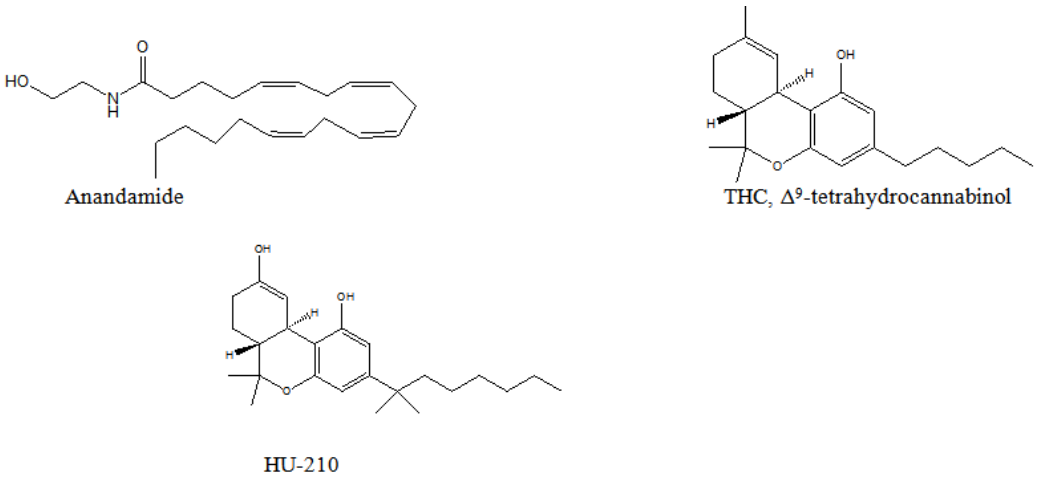


Fig 2: Cannabinoids

In the past several years, it has become apparent from preclinical studies that therapies either directly or indirectly influencing cannabinoid receptors might be clinically useful. The two known subtypes of cannabinoid receptors are CB1 and CB2. The CB1 receptor is mainly expressed in the immune cells, likely roles of these receptors include modulation of cytokine release and of immune cell migration.CB1 receptor ligands have therapeutic potential in a range of disorders including, obesity, nicotine addiction, pain and inflammation, gastrointestinal disorders, multiplesclerosis, psychosis, schizophrenia, osteoporosis.

The indole scaffold probably represents one of the most important structural subunits for the discovery of new drug candidates. The demonstration that many alkaloids contain the indole nucleus, the recognition of the importance of essential amino acid, tryptophan in human nutrition and the discovery of plant hormones served to bring about a massive search on indole chemistry, giving rise to a vast number of biologically active natural and synthetic products, with a wide range of therapeutic targets, such as anti-inflammatories, phosphodiesterase inhibitors, 5-hydroxytrytamine receptor agonists and antagonists, cannabinoid receptors agonists and HMG-CoA reductase inhibitors.

Allosteric modulation could be a new effective approach to manipulate the endocannabinoid system by enhancing the signaling of already existing selective agonists, inverse agonists and enzyme inhibitors. The allosteric modulation of the CB1 cannabinoid receptors promises to provide a ground breaking importance in drug design.

The analogues synthesized are structurally related to a pre-existing compound, F087 which is a CB1 receptor allosteric modulator (studies carried out by AstraZeneca, Adam et al, 2007).

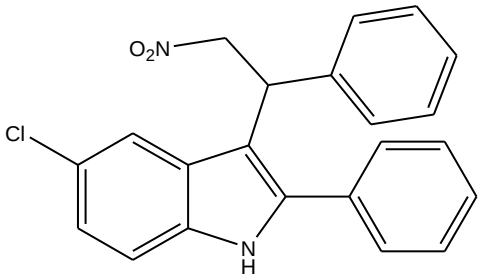
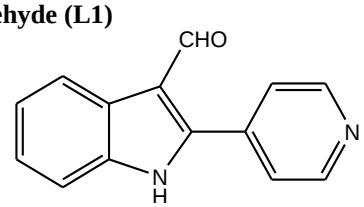


Fig. 3:F087

II. Material and Methods

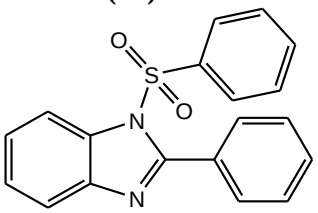
2-Pyridin -4-yl-1H-indole-3-carbaldehyde (L1)



A mixture of 2-pyridine indole (0.5g, 2.57 mmol, 1equiv.), DMF (0.3ml, 1.5 equiv.) and $POCl_3$ (0.3ml, 1.5 equiv.) were placed in a 50 ml round bottom flask. DCM was added and the mixture refluxed under an oil bath at 50^0C for 20 hrs. TLC using a mixture of ethyl acetate and hexane (1:2) was used to confirm completion of reaction. DCM was then removed using a rotary evaporator. Solid obtained (light yellow) was washed, dried and then mixed with sodium acetate slurry (8g of sodium acetate dissolved in 100ml of water). The mixture was stirred for 2 hrs. The resulting mixture was filtered under pressure, washed and dried with hexane. Product was purified by recrystallisation. Ethanol was used for recrystallisation and it was done thrice.

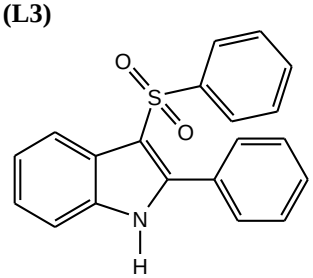
Bright yellow crystals (0.653g, 85%) were obtained. Melting point 132-135^{0}C, 1H NMR (400 MHz, DMSO): δ 10.04 (1H, s); 8.77-8.76 (2H, m); 8.23-7.79 (1H, m); 7.79-7.77 (2H, d); 7.53-7.52 (1H, d); 7.34-7.24 (2H, m). 13C NMR (100MHz, DMSO): δ 187.20; 150.20; 149.80; 145.70; 136.50; 128.50; 124.20; 122.30; 122.10; 121.70; 121.20; 120.30; 111.60; 101.80

1-Benzenesulfonyl-2-phenyl-1H-benzoimidazole (L2)



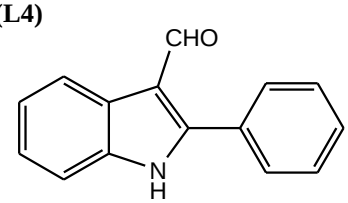
A mixture 2-phenylbenzimidazole (0.38g, 2 mmol), Benzenesulfonyl chloride (0.3ml, 2.5 mmol) and Indium tribromide (0.0042g, 10mol %) in Toluene (10ml) was refluxed at 115^{0}C for 8 hrs. Reaction mixture was then diluted with water (20 ml) and extracted using DCM. The combined organic layer was dried over Na_2SO_4, filtered under gravity and concentrated using a rotary evaporator. The product obtained (yellow coloured oil) was purified by flash chromatography using a mixture of ethyl acetate and hexane (1:4). Yellow oil (0.57g, 87%) was obtained. 1H NMR (400MHz, CDCl3): δ 8.2 (1H, m); 7.74 -7.60 (1H, m); 7.59-7.51 (2H, m); 7.50-7.38 (8H, m); 7.37-7.25 (2H, m). 13C (100MHz, CDCl3): δ 142.61; 137.91; 134.39 ; 133.90; 130.84; 130.56; 129.93; 129.09 ; 127.69; 126.90 ; 125.55 ; 125.38 ; 120.45 ; 115.15.

3-Benzenesulfonyl-2-phenyl-1H-indole (L3)



A mixture of 2-phenyl indole (1.15g, 6mmol), benzenesulfonyl chloride (0.9ml, 7.5mmol) and Indium tribromide (0.0042g, 10mol %) were placed in placed in a 50ml round bottom flask. 30 ml of Dichloroethane was added and mixture refluxed at 90^{0}C for 8 hrs. 40ml of distilled water was added to the reaction mixture and then extraction was done using Dichloromethane. The combined organic layer was dried over Na_2SO4 to remove any traces of water. TLC was then done to check extent of reaction. Desired product was not obtained considering the fact that there were multiple spots showing for the product obtained. The experiment was repeated twice and similar results obtained for each experiment.

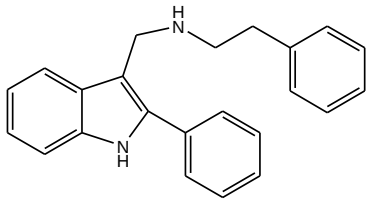
2-Phenyl-1H-indole-3-carbaldehyde (L4)



2-phenylindole (2g, 10.3mmol), DMF (1.2ml, 15.5mmol) and DCM (60ml) were placed in a 250ml two-necked flask. The mixture was stirred under a continuous purge of argon gas for 20 min. $POCl_3$ (1.2ml, 12.87mmol)was added drop wise over 2 min and then the reaction was refluxed for 20hrs at 50^{0}C. The reaction mixture was filtered under gravity to remove DCM and the residue slurried in sodium acetate (12g dissolved in

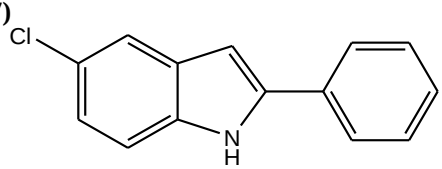
150ml of water) for 2hrs. The product was filtered off, washed with water and dried with hexane to obtain pure bright yellow crystals (2.01g, 89%).

Phenethyl-(2-phenyl-1H-indol-3-ylmethyl)-amine (L5)



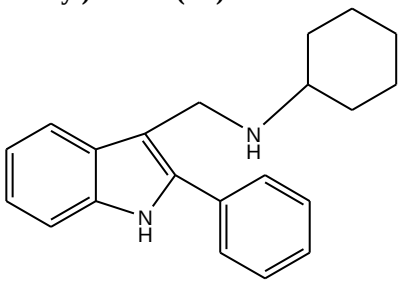
2-Phenyl-1H-indole-3-carbaldehyde, L4 (0.5g, 2.26mmol, 1equiv) and 2-Phenylethylamine (0.34ml, 2.71mmol, 1.2 equiv) were dissolved in Ethanol (30ml) and the mixture refluxed overnight at 87^0C. After cooling, Sodium cyanoborohydride $NaCNBH_4$ (0.17g) was added to the reaction mixture and stirred for 30 min at room temperature. Distilled water (30ml) was added and the reaction mixture concentrated to a minimum using a rotary evaporator. The product was extracted with Ethyl acetate (100ml) and the organic layer dried over $MgSO_4$. The mixture was concentrated in vacuum to obtain orange coloured oil which was dissolved in 20ml of Ethyl acetate and treated with 3M HCl. The mixture was allowed to stand for 6 hrs to allow for complete formation of precipitate. The precipitate was filtered under pressure, washed with Ethyl acetate and dried to give a secondary amine as a hydrochloride salt. The salt was dissolved in water/methanol (20ml: 20ml) and treated with saturated solution of $NaHCO_3$ till alkaline medium was obtained to liberate free amine (use litmus paper was used to check for PH range of 9-11). The solution was concentrated to a minimum using a rotary evaporator and extracted with ethyl acetate (50ml). The organic layer was dried over MgSO4 and concentrated in vacuum to obtain orange coloured oil. The product was purified by column chromatography on silica gel (ethyl acetate – hexane, 1:4). Pure product obtained as a bright yellow oil (0.47g, 65%). 1H NMR (400MHz, CDCl3): 8.2 – 8.1 (2H, m); 7.64 -6.73 (16H, m); 3.99 (2H, d); 2.95-2.65 (4H, m). ^{13}C (100MHz, CDCl3): δ 128.98; 128.90 ; 128.72 ; 128.61; 128.41; 128.27; 128.14; 128.02 ; 127.93; 127.56; 126.11; 122.43; 122.16; 120.01; 119.73; 119.01; 110.86; 110.60; 77.35; 77.03; 76.71; 50.49; 43.47; 35.81.

5-Chloro-2-phenyl-1H-indole (L7)



4-Chlorophenylhydrazine hydrochloride (1g, 5.58mmol, 1 equiv) and ethanol (15ml) were placed in a 150ml round bottom flask. Acetophenone (0.652ml, 5.58mmol, 1 equiv) was added and the reaction mixture stirred overnight at reflux temperature (90^0C). The mixture was then concentrated using a rotary evaporator, washed and dried to obtain a hydrazone (acetophenone4-chlorophenylhydrazone). Polyphosphoric acid (17g) and toluene (20ml) were added to the hydrazone and the mixture refluxed at 120^0C for 2 hrs. Toluene was then removed from the mixture. The reaction flask was placed in an oil bath and temperature set to 100^0C, then distilled water (enough to cover the polyphosphoric acid) was added and the mixture stirred at 100^0C for an hr. Reaction mixture was filtered, washed with water and dried to obtain brown solid (0.95g, 73%).

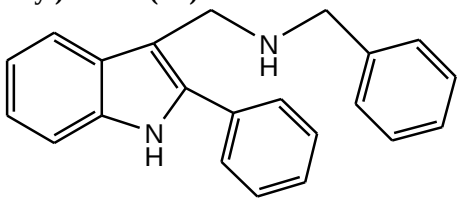
Cyclohexyl-(2-phenyl-1H-indol-3-ylmethyl)-amine (L8)



2-Phenyl-1H-indole-3-carbaldehyde, L4 (0.5g, 2.26mmol, 1equiv) and cyclohexylamine (0.31ml, 2.70mmol, 1.2 equiv) were dissolved in Ethanol (30ml) and the mixture refluxed overnight at 87^0C. After cooling, Sodium cyanoborohydride $NaCNBH_4$ (0.17g) was added to the reaction mixture portion wise over 20

min and stirred for 30 min. at room temperature. Distilled water (30ml) was added and the reaction mixture concentrated to a minimum using a rotary evaporator. The product was extracted with Ethyl acetate (100ml) and the organic layer dried over $MgSO_4$. The mixture was concentrated in vacuum to obtain orange coloured oil which was dissolved in 20ml of Ethyl acetate and treated with 3M HCl until formation of precipitate. The precipitate was filtered under pressure, washed with Ethyl acetate and dried to give a secondary amine as a hydrochloride salt. The salt was dissolved in water/methanol (20ml: 20ml) and treated with saturated solution of $NaHCO_3$ till alkaline medium was obtained to liberate free amine (use litmus paper was used to check for PH range of 9-11). The solution was concentrated to a minimum using a rotary evaporator and extracted with ethyl acetate (50ml). The organic layer was dried over $MgSO_4$ and concentrated in vacuum to obtain orange colored oil. The product was purified by column chromatography on silica gel (ethyl acetate – hexane, 1:2). Pure product obtained as a bright yellow oil (0.46g, 68%). 1H NMR (400MHz, CDCl3): δ8.10-7.88 (2H, d); 7.29-6.12 (9H, m); 4.85 (1H, s), 3.87-2.03 (5H, m). ^{13}C (100MHz, $CDCl_3$): δ 128.81; 128.46; 128.16; 127.79; 122.27; 119.81; 118.70; 111.14; 56.75; 40.80; 33.33; 31.68; 26.17; 25.13; 25.00; 24.51.

Benzyl-(2-pheny-1H-indol-3-ylmethyl)-amine (L9)



2-Phenyl-1H-indole-3-carbaldehyde, L4 (0.5g, 2.26mmol, 1equiv) and cyclohexylamine (0.30ml, 2.71mmol, 1.2 equiv) were dissolved in Ethanol (30ml) and the mixture refluxed overnight at 87^0C. After cooling, Sodium cyanoborohydride $NaCNBH_4$ (0.17g) was added to the reaction mixture portion wise over 20 min and stirred for 30 min at room temperature. Distilled water (30ml) was added and the reaction mixture concentrated to a minimum using a rotary evaporator. The product was extracted with Ethyl acetate (100ml) and the organic layer dried over $MgSO_4$. The mixture was concentrated in vacuum to obtain orange coloured oil which was dissolved in 20ml of Ethyl acetate and treated with concentrated HCl until formation of precipitate. The precipitate was filtered under pressure, washed with Ethyl acetate and dried to give a secondary amine as a hydrochloride salt. The salt was dissolved in water/methanol (20ml: 20ml) and treated with saturated solution of $NaHCO_3$ till alkaline medium was obtained to liberate free amine (use litmus paper was used to check for PH range of 9-11). The solution was concentrated to a minimum using a rotary evaporator and extracted with ethyl acetate (50ml). The organic layer was dried over $MgSO_4$ and concentrated in vacuum to obtain orange colouredoil. The product was purified by column chromatography on silica gel (ethyl acetate – hexane, 1:2). Pure product obtained as bright yellow oil (0.50g, 72%).

III. Results

The pyridine indole/ phenyl indole (1) was treated with dimethylformamide Phosphorus oxytrichloride using Dichloromethane as solvent and the mixture refluxed to afford the corresponding indolecarbaldehyde (L1/L4).

Sulphonation of 2-phenylbenzimidazole (2) was performed using benzenesulfonyl chloride, indium tribromide as a catalyst and toluene as the solvent to obtain Benzene sulfonyl-2-phenylbenzoimidazole, L2 (Scheme 2).

Scheme 2 above was repeated using 2-phenylindole (4), Benzenesulfonyl chloride (5), Indiumtribromide as catalyst and Toluene as solvent, however the desired product was not obtained. TLC for the reaction mixture showed two spots relatively close to each other. This suggests that the sulphonation of the indole might have occurred in two different positions (Scheme 3).

Phenyl indolecarbaldehyde (6) was treated with an amine (7) in the presence of Ethanol to yield the amine derivatives, (8). Reduction using $NaBH_4CN$ afforded the corresponding Phenyl indole amine, L5, L8, L9, (Scheme 5). A good yield was obtained for the three compounds (65, 68 and 72% respectively).

The hydrazine hydrochloride (9) was treated with acetophenone (10) to afford the hydrazone which was further treated with poyphosphoric acid (a bronsted acid) in the presence of toluene to yield 5-chloro-2-phenylindole, L7 (Scheme 5).The reaction proceeded with ease however specific precaution is taken during the conversion of the hydrazone to the indole, boiling water is added to the mixture after removal of toluene to ensure that good crystals of the indole is obtained.

The syntheses performed so far were done with ease although some precautions were required for specific reactions. The indoles generally undergo electrophilic substitution with ease. Some of the compounds have shown biological activity as CB1 allosteric modulators (L2 and L5), the others are yet to be tested for

biological activity. Generally it can be assumed that a phenyl group on the 2-position of the indole ring is essential for a compound to act as an allosteric modulator of the CB1 cannabinoid receptors. Also the Benzoimidazole ring might be a good candidate for this same purpose.

IV. Conclusion

The indole scaffold probably represents one of the most important structural subunits for the discovery of new drug candidates. The demonstration that many alkaloids contain the indole nucleus, the recognition of the importance of essential amino acid tryptophan in human nutrition and the discovery of plant hormones served to bring about a massive search on indole chemistry, giving rise to a vast number of biologically active natural and synthetic products, with a wide range of therapeutic targets, such as anti-inflammatories, phosphodiesterase inhibitors, 5-hydroxytryptamine receptor agonists and antagonists, cannabinoid receptors agonists and HMG-CoA reductase inhibitors.

Many of these target receptors belong to the class of GPCRs (integral membrane G-protein coupled receptors) and possess a conserved binding pocket that is recognized by the indole scaffold in a "common" complementary binding domain, explaining the great number of drugs that contain the indole substructure, such as indomethacin, ergotamine, frovatriptan, ondansetron, tadalafil, among many others.

The allosteric modulation of the CB1 cannabinoid receptors promises to provide a ground breaking importance in drug design. Allosteric modulators of cannabinoid CB1 receptors would offer the prospect of producing clinically useful compounds free of any CNS side effects.

Future intended work includes the following;

- Synthesis of derivatives of compounds (L2, L5) by introducing and interchanging functional groups.
- Synthesis of amine derivatives of L1
- Sulfonation and amination of 5-chloro-2-phenyl indole
- Parallel synthesis using the microwave

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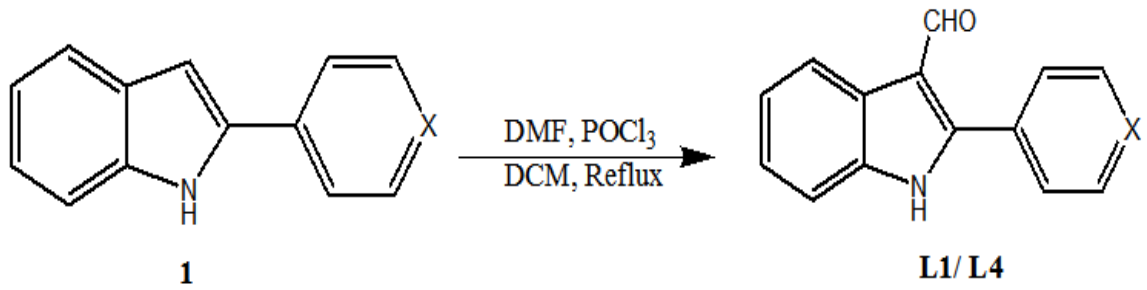
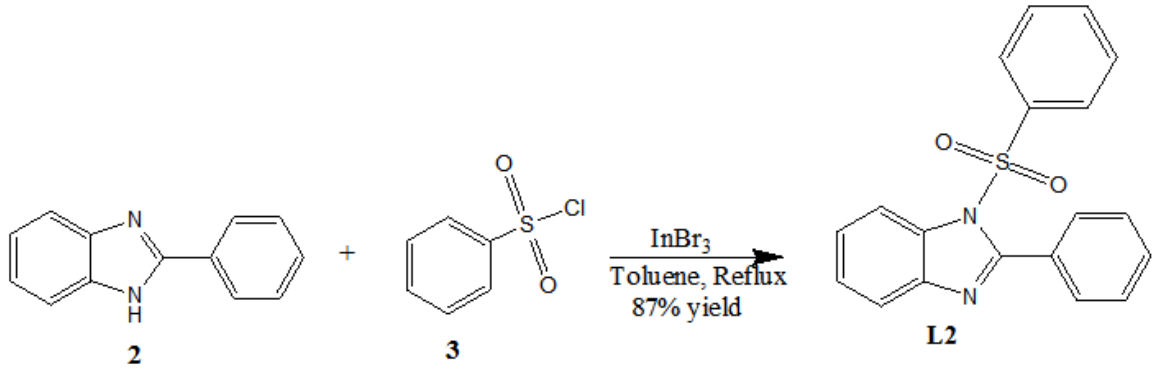
Scheme 1



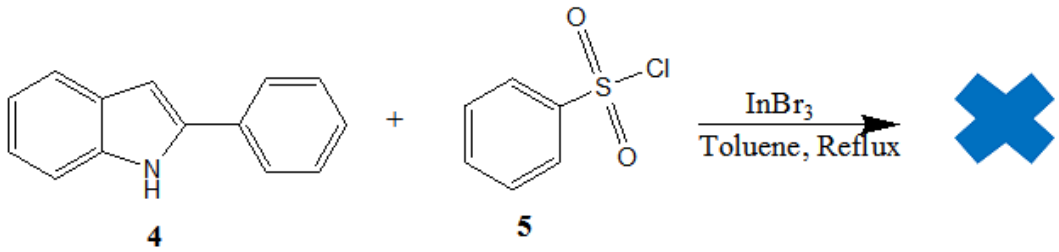
1 **L1/ L4**

X = N, CH

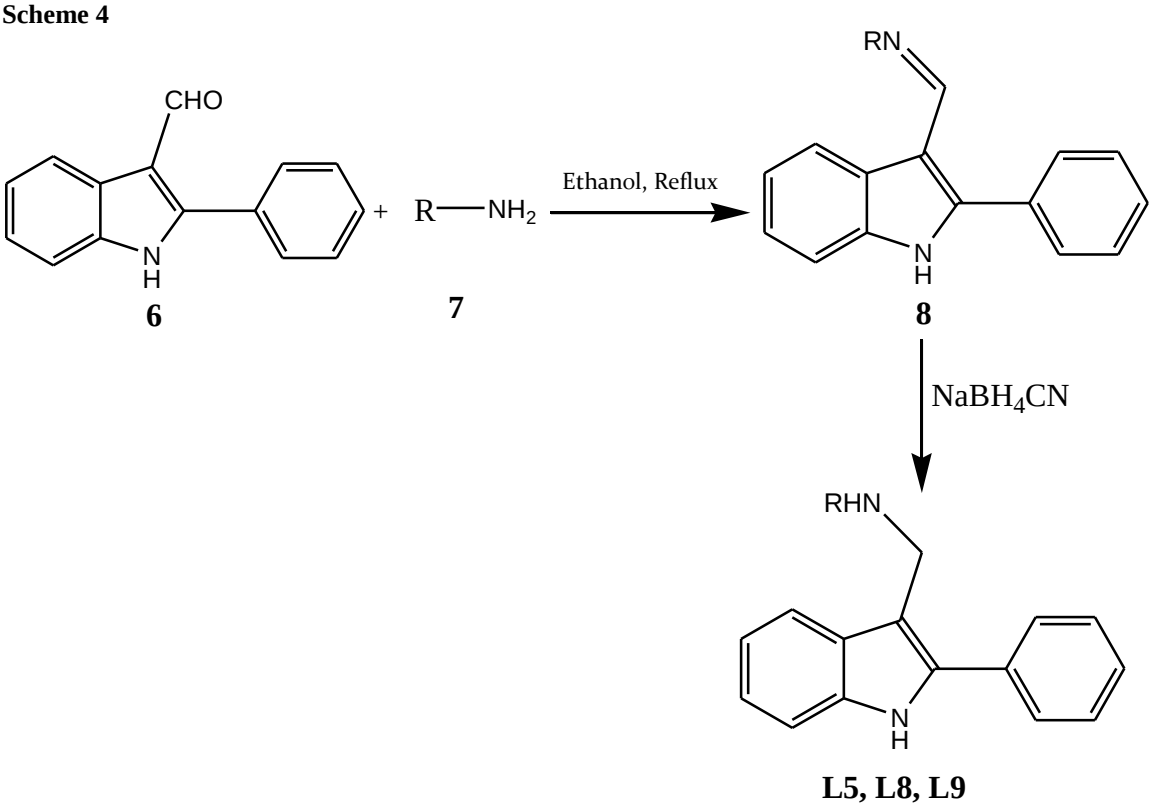
Scheme 2



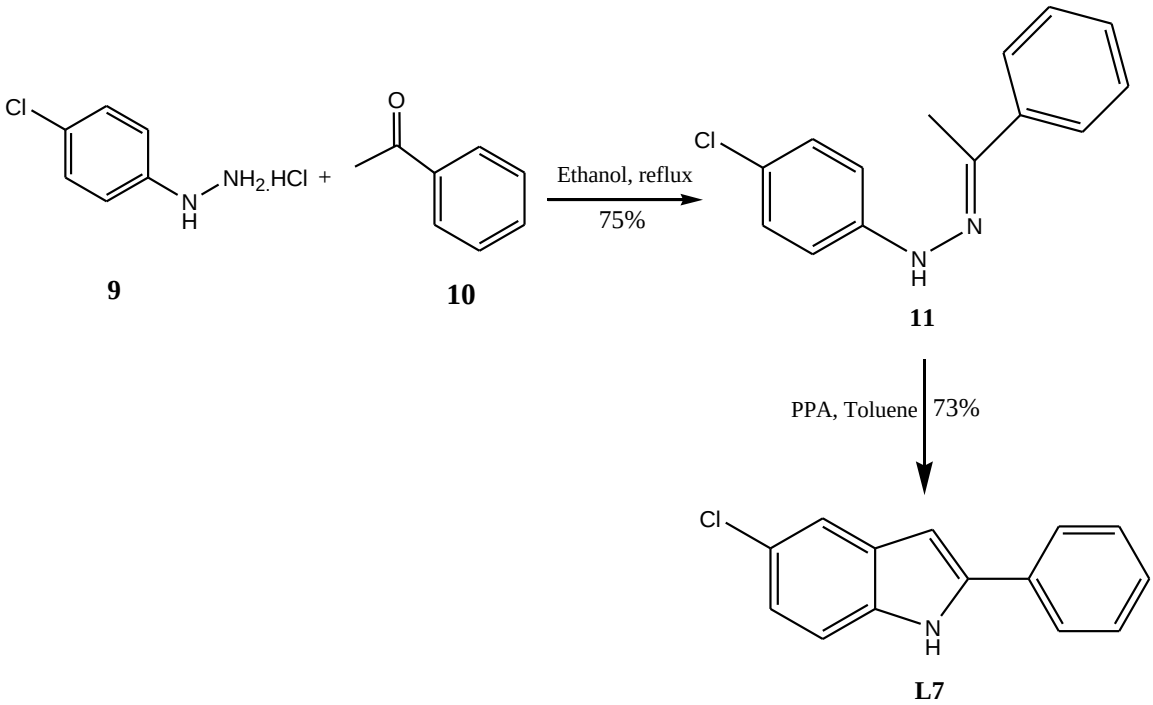
Scheme 3



Scheme 4



Scheme 5



9 **10** **11** **L7**

Ugwuonah Linda .A.. " Synthesis of Allosteric Modulators of CB1 Cannabinoid Receptors." IOSR Journal of Applied Chemistry (IOSR-JAC) 11.6 (2018): 63-70.