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Synthesis and in Vitro Cytotoxicity Evaluation of Isatin-Pyrrole Derivatives against HepG2 Cell Line

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ABSTRACT

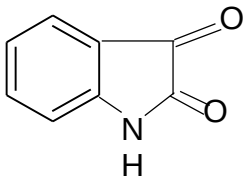
This paper reports the synthesis and *in vitro* cytotoxicity evaluation of isatin-pyrrole derivatives 5–8, obtained from the appropriate isatins with pyrrole, with good yields and purity. The product structures were confirmed through spectroscopy methods. Furthermore, the MTT assay on the human liver cancer HepG2 cell lines revealed moderate activity in all compounds, which was highest in sample 6 (IC50 0.47 μM). The anticancer activity was affiliated with the presence of a nitro group at C-5 and *N*-methyl of the isatin scaffold.

Keywords : isatin; isatin-pyrrole derivatives; anticancer; HepG2 cell line.

I. INTRODUCTION

Cancer is a serious threat to human health and a leading cause of death globally [1]. According to the GLOBOCAN 2018 database, 18.1 million people of all ages have various types of cancer, leaving almost half of the total affected individuals dead [2]. Additionally, chemotherapy is one of the most common treatments and is known to confer several disadvantages, including toxicity to normal cells [3]. Hence, there is a need to develop drugs with lower cytotoxicity. Moreover, the most promising approach is molecular hybridization or pharmacophore hybrid [4]. This involves the combination of two distinct pharmacophore functions to produce synergistic, more powerful, selective, and safer drugs [5].

Great efforts have been made to promote this technique, based on the isatin skeleton to develop cancer drugs [6], and recent studies show good anticancer activity in isatin **1** and its derivatives [7]. For example, the isatin-podophyllotoxin and nitroimidazole-isatin hybrid were reported to be active against human leukaemia and breast cancer cells, respectively [8,9]. These effects are altered by modifications at the C-3, amide group, and phenyl ring of the isatin hybrid, as better activity was detected with the presence of a nitro group at C-5 isatin and a methyl or benzyl group at N-isatin [10,11,12].



Pyrrole is another important active chromophore with heterocyclic aromatic characteristics. It contains a nitrogen atom and is part of the cofactors and natural products of vitamin B12 and porphyrinogens [13,14]. Furthermore, pyrrole possesses broad-spectrum bioactivities, including anticancer and antibacterial functions [15,16], while molecular hybrid derivatives, including oroidin and sophoridine, recently exhibited remarkable anticancer activity against MCF-7 and HepG2 cancer cell lines [15,17]. Moreover, the trimethoxybenzaldehyde-pyrrole hybrid demonstrated good effects against HeLa and MCF-7 [18].

These findings suggest the need to investigate the combination of pharmacophoric elements including isatin and pyrrole, in a single chemical framework, and to investigate their cytotoxicity. Additionally, the effect of the nitro and amino group at the C-5 region of isatin and the methyl group of N-isatin on the compound's anticancer activity was also investigated. This study, therefore, reports on the synthesis of isatin-pyrrole derivatives alongside with their anticancer activity against HepG2 cancer cell lines.

Materials and method

All chemicals and solvents were purchased from commercial suppliers and used without purification. Melting points were measured using a Fisher John apparatus and are uncorrected. The Fourier-Transform Infrared (FT-IR) spectrum was confirmed using FTIR spectrophotometer Shimadzu 8400S. The mass spectra were recorded using LC-MS Mariner Biospectrometry Hitachi L 6200 with ESI Waters LCT Premier XE or TOF-MS Waters LCT Premier XE mass spectrometer. The proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) were measured in acetone-d_6 solvent using FT-NMR JNM-ECA500 500 MHz and FT-NMR JNM-ECS400 400 MHz.

1. Synthesis of N-methyl-5-nitroisatin (3)

The synthesis of **3** was performed by stirring 5-nitroisatin **(2)** (200 mg, 1.04 mmol) and sodium hydride (100 mg, 4.16 mmol) in anhydrous dimethyl sulfoxide (10 mL) at rt for 1 h. Dimethyl sulfate (0.40 mL, 4.16 mmol) was added, and the mixture was cooled with ice with stirring for 2 h before adding cold water. The resulting precipitate was filtered off, washed with water, and dried to yield N-methyl-5-nitroisatin **(3)** as a yellow solid (200 mg, 95%), mp 145–146°C (lit. 132–134°C [19]). IR (KBr) ν cm^{-1}: 3,063 (C–H aromatic), 2,945 (C–H sp^3), 1,743 (C═O), and 1,608 cm^{-1} (C═C aromatic). ^{1}H-NMR (500 MHz, acetone-d_6): δ, ppm 3.36 (s, 3H, CH_3), 7.41 (d, J = 9.1 Hz, 1H, ArH), 8.32 (d, J = 2.6 Hz, 1H, ArH), and 8.60 (dd, J = 9.1, 2.6 Hz, 1H, ArH).

2. Synthesis of 3-hydroxy-3-(1H-pyrrol-2-yl)indolin-2-one (5)

A solution of isatin **(1)** (0.15 g, 1.02 mmol) in methanol:water (1:1) (20 mL) was stirred at 50°C, and then potassium carbonate (7.0 mg, 0.051 mmol) and pyrrole **(4)** (71 µL, 1.02 mmol) were added. After stirring for 30 h, cold water was then incorporated, and the product was extracted several times with dichloromethane. The extracts were combined, dried over magnesium sulfate, followed by evaporation under reduced pressure. Subsequently, the crude product was purified using column chromatography with chloroform:ethyl acetate (3:1) eluant to yield 3-hydroxy-3-(1H-pyrrol-2-yl)indolin-2-one **(5)** as a black solid (100 mg, 45%), mp 151–152°C. IR (KBr) ν cm^{-1}: 3,375 (N–H), 3,198 (O–H), 1,710 (C═O), and 1,622 cm^{-1} (C═C aromatic). ^{1}H-NMR (500 MHz, acetone-d_6): δ, ppm 5.37 (s, 1H, O–H), 5.67 (1H, d, ArH pyrrole), 5.90 (t, 1H, ArH pyrrole), 6.80 (d, 1H, ArH pyrrole), 6.89 (1H, d, ArH isatin), 7.02 (1H, t, ArH isatin), 7.23 (1H, t, ArH isatin), 7.27 (1H, d, ArH isatin), 9.26 (bs, 1H, N–H isatin), and 10.04 (bs, 1H, N–H pyrrole). ^{13}C-NMR (125 MHz, acetone-d_6): δ, ppm 73.3, 107.1, 107.4, 110.0, 119.4, 122.0, 125.2, 129.4, 129.8, 131.8, 141.9, and 177.3. HRMS (ESI): m/z calcd for $C_{12}H_9N_2O_2$, $[M - H]^+$ 213.2121; found: 213.1722.

3. Synthesis of 3-hydroxy-5-nitro-3-(1H-pyrrol-2-yl)indolin-2-one (6)

A solution of 5-nitroisatin (**2**) (72 mg, 0.37 mmol) in methanol:water (1:1) (10 mL) was stirred at 50°C, and then potassium carbonate (2.6 mg, 0.019 mmol) and pyrrole **(4)** (0.026 µL, 0.37 mmol) were added. After stirring for 5 h, cold water was added, and the product was extracted several times with dichloromethane. These combined extracts were dried over anhydrous magnesium sulfate and evaporated under reduced pressure to produce 3-

hydroxy-5-nitro-3-(1H-pyrrol-2-yl)indolin-2-one **(6)** as a green solid (49 mg, 51%), mp 163–164°C. IR (KBr) ν cm^{-1}: 3,375 (N–H), 3,279 (O–H), 1,720 (C═O), and 1,627 cm^{-1} (C═C aromatic). ^{1}H-NMR (500 MHz, acetone-d $_6$): δ, ppm 5.82–5.83 (m, 1H, ArH pyrrole), 5.84 (bs, 1H, OH), 5.97–5.99 (m, 1H, ArH pyrrole), 6.89–6.90 (m, 1H, ArH pyrrole), 7.18 (d, J = 9.1 Hz, 1H, ArH isatin), 8.28 (dd, J = 9.1, 2.6 Hz, 1H, ArH isatin), 8.36 (d, J = 2.6 Hz, 1H, ArH isatin), 9.95 (bs, 1H, NH pyrrole), and 10.30 (bs, 1H, NH isatin). ^{13}C-NMR (125 MHz, acetone-d $_6$): δ, ppm 74.9, 108.6, 108.8, 111.3, 121.3, 121.9, 127.0, 129.0, 134.3, 144.9, 149.1, and 180.3. HRMS (ESI): m/z calcd for $C_{12}H_{10}N_3O_4$, $[M + H]^+$ 260.0671, found: 260.0674.

4. Synthesis of 3-hydroxy-N-methyl-5-nitro-3-(1H-pyrrol-2-yl)indolin-2-one (7)

A solution of N-methyl-5-nitroisatin (**3**) (110 mg, 0.53 mmol) in methanol:water (1:1) (20 mL) was stirred at 50°C, and then potassium carbonate (5.37 mg, 0.039 mmol) and pyrrole **(4)** (26 µL, 0.37 mmol) were added. After stirring for 2 h, cold water was added, and the product was extracted several times with dichloromethane. These extracts were dried over magnesium sulfate and evaporated under reduced pressure to generate 3-hydroxy-5-nitro-3-(1H-pyrrol-2-yl)indolin-2-one **(7)** as a green solid (88 mg, 63%), mp 139–140°C (lit. 102–103°C [20]). IR (KBr) ν cm^{-1}: 3,543 (N–H), 3,325 (O–H), 1,710 (C═O), and 1,614 cm^{-1} (C═C aromatic). ^{1}H-NMR (500 MHz, acetone-d $_6$): δ, ppm 3.27 (s, 3H, CH_3), 5.81–5.82 (m, 1H, ArH pyrrole), 5.89 (bs, 1H, OH), 5.96–5.98 (m, 1H, ArH pyrrole), 6.88–6.89 (m, 1H, ArH pyrrole), 7.25 (d, J = 7.8 Hz, 1H, ArH isatin), 8.34–8.37 (m, 2H, ArH isatin), and 10.32 (bs, 1H, NH pyrrole). ^{13}C-NMR (125 MHz, acetone-d $_6$): δ, ppm 29.9, 73.8, 108.1, 108.4, 109.6, 121.0, 121.1, 127.3, 129.3, 133.3, 144.3, 150.3, and 176.9. HRMS (ESI): m/z calcd for $C_{13}H_{12}N_3O_4$, $[M + H]^+$ 274.2521, found: 274.2660.

5. Synthesis of 5-amino-3-hydroxy-3-(1H-pyrro-2-yl)indolin-2-one (8)

5-Amino-3-hydroxy-3-(1H-pyrrol-2-yl)indolin-2-one **(8)** was produced by reducing 3-hydroxy-5-nitro-3-(1H-pyrrol-2-yl)indolin-2-one **(6)**. This reduction involved heating a mixture of **6** (75 mg, 0.29 mmol) and Pd/C (7 mg) in ethanol (10 mL) at reflux for 1 h. Then, hydrazine hydrate (40 equiv) was added dropwise, followed by heating at reflux for an additional 1 h and subsequently filtered after cooling. The filtrate was evaporated under reduced pressure, followed by crude product purification using column chromatography with chloroform:ethyl acetate (1:3). Then **8** was generated as a brown solid (31 mg, 47%), mp 170–171°C. IR (KBr) ν cm^{-1}: 3,365 (NH_2), 3,225 (O–H), 1,703 (C═O), and 1,624 cm^{-1} (C═C aromatic). ^{1}H-NMR (500 MHz, DMSO-d $_6$): δ, ppm 4.80 (2H, bs, NH_2), 5.59 (1H, s, OH), 5.91 (1H, d, ArH pyrrole), 5.90–5.91 (m, 1H, ArH pyrrole), 5.99–6.00 (m, 1H, ArH pyrrole), 6.68–6.79 (m, 1H, ArH pyrrole), 7.23 (d, J = 9.0 Hz, 1H), 8.26 (dd, J = 9.0, J = 2.6 Hz, 1H, ArH isatin), 8.36 (d, J = 2.6 Hz, 1H, ArH isatin), 9.88 (bs, 1H, NH isatin), and 10.76 (bs, 1H, NH pyrrole). ^{13}C-NMR (125 MHz, DMSO-d $_6$): δ, ppm 74.3, 106.8, 107.0, 110.4, 112.7, 114.3, 119.3, 131.1, 131.7, 133.5, 144.3, and 177.7. HRMS (ESI): m/z calcd for $C_{12}H_{10}N_3O_2$, $[M + Na]^+$ 252.2224, found: 252.1999.

6. Cell culture conditions

The HepG2 cell line was obtained from the Agency for Assessment and Application of Technology, Indonesia. The cells were routinely maintained and grown at 37°C, 5% CO_2 in a 95% humidified atmosphere. Additionally, the growth medium was prepared from Roswell Park Memorial Institute (RPMI) 1640 (Gibco) using phenol red, 2 mM glutamine, 100 U/mL penicillin, 0.1 mg/mL streptomycin, 1 mM sodium pyruvate, and 10% foetal bovine serum (FBS), which was previously inactivated at 56°C for 30 min. Cell passaging was performed using 4 mL of trypsin-EDTA at room temperature for 3 minutes. A total of 10 mL of media with 10% FBS was then used to reduce the action of trypsin on cells, and the resulting cells were plated after centrifugation.

7. Preparation of cytotoxicity test solutions:

The stock solutions of **5–8** and the doxorubicin control compound were individually combined with dimethyl sulfoxide (DMSO) and diluted serially in RPMI to yield the varying concentrations (12.5, 25, 50, 100, 200, and 400 μg/mL). A final concentration of 0.1% DMSO was obtained in the medium, and this was also used in the corresponding control. Additionally, no serum or antibiotics were introduced to the test and control mediums. All solutions were freshly prepared and protected from light.

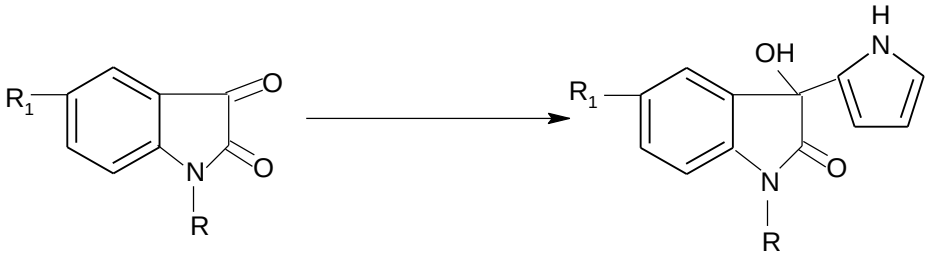
8. Cytotoxicity test

The cytotoxicity test was performed using the MTT method [21]. The HepG2 cells were maintained as monolayer cultures in RPMI 1640 medium and supplemented with antibiotics, including 100 IU/mL penicillin and 100 μg/mL streptomycin, and 10% FBS in a humidified incubator containing 5% CO_2 at 37°C. The subcultures were obtained by trypsin treatment of confluent cultures, and the resulting suspension (100 μL) (5×10^4 cells) was transferred to 96 well plates. These were then incubated in a CO_2 incubator for 24 h. The cell culture medium in each well was discarded and replaced with 100 μL of test solutions at various concentrations or the positive control (DMSO) before incubation for 24 h. Phosphate-buffered saline solution and 100 μL of MTT (0.5 mg/mL) were added to the wells, and the cells were incubated for an additional 4 h until blue coloured formazan crystals were observed. Subsequently, a 10% solution of sodium dodecyl sulfate in 0.1 N HCl was added, and the cells were incubated for the next 4 h at room temperature. The absorbance was measured using an ELISA plate reader at 570 nm, and the percentage of cell viability was then calculated. The IC_{50} value was determined by plotting the percentage of cell viability against sample concentration, and the assay was performed in triplicate.

Ethical approval: The conducted research is not related to either human or animal use.

Results and discussion

N-Methyl-5-nitroisatin (**3**) was synthesized using techniques from previous works [22,23,24]. This synthesis involved a reaction between 5-nitroisatin (**1**) and sodium hydride and dimethyl sulfoxide, followed by a reaction with dimethyl sulfate to generate the yellow solid N-methyl-5-nitroisatin (**2**) (Scheme 1). Subsequently, the structure of **3** was confirmed with (1) FT-IR, where the spectrum showed peaks at 3,063, 2,945, 1,743, and 1,608 cm^{-1} designating C–H aromatic, C–H sp^3, C═O, and C═C aromatic groups, respectively. (2) In ^{1}H-NMR, the spectrum showed a singlet at 3.55 ppm which indicated methyl group protons and two doublets at 7.41 and 8.32 ppm and another doublet at 8.60 ppm for aromatic protons. A previous report [25] showed the presence of singlet signal at 3.38 ppm for methyl group protons, based on ^{1}H-NMR data (in $CDCl_3$). However, the chemical shift reported in this research at 3.55 ppm due to measurement was carried out in different solvents (in acetone-d6); and the absence of NH proton signal in the NMR data suggests the successful synthesis of compound **3**.



1.R=H , R_1=H

2.R=H , R_1=NO_2

3.R=Me , R_1= NO_2

4. R=H , R_1=H

5. R=H , R_1=NO_2

6. R=Me , R_1=NO_2

Scheme 1

Synthesis of **3**. Reagents/conditions: (a) (i) NaH (4 eq), DMSO, rt 1 h; (ii) DMS (4 eq), cold 2 h, 89%

The isatin-pyrrole derivatives **5–8** were prepared through a reaction between the appropriate isatins **1–3** and pyrrole **(4)**, using method from previous work for indoles [26]. This process was initiated by dissolving the isatins in methanol:water, followed by the introduction of potassium carbonate as a catalyst. Then pyrrole **(4)** was added to obtain the final derivative products **5–8** (Scheme 2). The yields were of acceptable purity and were further subjected to analysis using FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry. Additionally, the FT-IR spectra showed peaks at the 3,375–3,424 cm^{-1} region, indicating an N–H group, at 3,198–3,325 cm^{-1} for an O–H group, at 1,703–1,720 cm^{-1} for a carbonyl group, and at 1,624–1,627 cm^{-1} for a C=C aromatic. The ^{1}H-NMR spectra showed greater deshielding in the NH pyrrole than the NH isatin, and the inverse was the case with the aromatic protons. This was due to the relatively lesser aromatic characteristics of pyrrole. The isatin-pyrrole derivative **7** showed a singlet resonance in its ^{1}H NMR at 3.27 ppm (in acetone-d_6) for the methyl group protons, which is similar with Li et al. data [20] at 3.25 ppm (in $CDCl_3$). Moreover, ^{13}C NMR spectra exhibited peaks corresponding to quartener carbons (C-3) at 73.3–74.9 ppm, carbonyls at 176.9–180.3 ppm, and the quaternary aromatic carbons were less deshielded than the tertiary form. The treatment of isatin derivative **(6)** with hydrazine and palladium on charcoal in ethanol led to the production of compound **(8)**, following nitro group reduction method of previous work [27]. This exhibited an FT-IR spectrum with an NH_2 peak at 3,543 and 3,423 cm^{-1} for unsymmetrical and symmetrical N–H, respectively.

Scheme 2 Synthesis of **5–8**. Reagents/conditions: (a) **4** (1 eq), MeOH:H_2O (1:1), K_2CO_3, 50°C 2–30 h, **5** (45%), **6** (51%), **7** (63%); (b) (i) Pd/C, EtOH, reflux 1 h; (ii) $NH_2NH_2.H_2O$ (40 eq), EtOH, reflux 1 h,

The cytotoxicity test of isatin derivatives **(5–8)** against the liver cancer cell line HepG2 was performed using a colorimetric method. This method was based on the ability of mitochondrial dehydrogenase enzyme to convert 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to formazan, indicated by a colour change from yellow to blue. Furthermore, the result was analysed using an ELISA reader, and the IC_{50} values of **5–8** against HepG2 cells are shown in Table 1. The results showed the ability for substitutions at C-5 and the presence of N-methyl on the isatin scaffold to influence bioactivity. Meanwhile, isatin-pyrrole **6** bearing a nitro group at C-5 of the isatin scaffold was identified as the most active compound, due to the IC_{50} of 0.47 μM, although the N-methyl group tends to reduce the effect.

Table 1. Anticancer activity of synthesized compounds

Compounds	IC_{50} (μM)
5	10.33
6	0.47
7	1.33
8	4.64
Doxorubicin	0.00035

Table 2. In vitro Antibacteria activities of the N-methyl isatin and its derivatives (*MIC's in ug/ml)

Microorganism/drugs	C-1	C-2	C-3	C-4	TM	SM	NFX
Salmonella paratyphi A	156.25	312.25	312.5	78.125	156.25	2500	9.76
Staphylococcus aureus	625	5000	1250	>5000	>5000	5000	2500
Vibrio cholerae 01 ogawa	1250	2500	312.5	5000	5000	5000	<0.01
Klebsiellapneumoniae	>5000	5000	2500	5000	5000	2500	1250
Shigelladysentriae	5000	2500	1250	5000	-	2500	1.22
Plesiomonasshigelliods	>5000	156.25	312.5	156.5	4.88	5000	9.76
Salmonella paratyphi B	5000	2500	2500	5000	9.76	5000	<0.01
Morganellamorganii	5000	625	312.5	78.125	156.25	2500	2.44
Edwardsiellatarda	156.25	312	312.5	15625	312.5	5000	9.76
Shigellaboydii	78.125	156.25	312.5	156.25	9.76	2500	<0.01
Shigellaflexneri	78.125	78,125	3125	1250	156.25	2500	2.44
Salmonella enteritidis	5000 ,	2500	2500	5000	4.88	2500	<0.01
Aeromonashydrophila	5000	2500	4250	>5000	1250	2500	0.3
Enterobacter	5000	625	1250	2500	156.25	1250	<0.01
Staphylococcus aureusATCC 225923	625	2500	312.5	5000	>5000	5000	250
Escherichia coli ATCC 292122	625	1250	2500	5000	19.53	2500	1.22
Shigellasonnei	1250	1250	2500	5000	9.76	2500	9.76
Vibrio mimicus	78.125	312.5	2500	5000	-	2500	0.1
Vibrio cholerateinaba	5000	5000	2500	2500	312.5	5000	0.1
Proteus mirabilis	>5000	5000	2500	>5000	156.25	2500	<0.01
Citrobacterferundii	5000	5000	2500	>5000	19.53	5000	<0.01
Salmonella typhimurium	5000	2500	2500	2500	>5000	5000	0.
Enterococcus faecalis	5000	5000	2500	22500	78.12	5000	9.76
Pseudomonas aeruginosa	5000	2500	2500	2500	5000	78.425	19
Escherchia coli	5000	5000	2500	5000	19.53	2500	1

MIC – Minimum inhibitory concentration (-) activities not found

Conclusions

Total of four isatin-pyrrole derivatives **(5–8)** were successfully synthesized in good yield and purity, and the structure was confirmed using FTIR, NMR, and MS. These products were tested for anticancer activity using the liver cancer cell line HepG2, and their IC_{50} values were calculated. The cytotoxicity assay of all compounds showed moderate action, although (**6)** exhibited the highest effect, with an IC_{50} of 0.47 μM.

References:

1. Sridhar SK, Pandeya SN, Stables JP, Ramesh A(2002)Anticonvulsant activity Of hydrazones, Schiff and Mannich bases of isatin derivatives. European Journal of Pharmaceutical Science 16(3): 129-132
2. Verma M, Pandeya SN, Singh KN, Stables(2004)JP. Acta 54: 36-49
3. Pandeya SN , S D Natgh G,de Clereq E(2000) Synthesis of antifungal and anti HIV evaluation of Schiff and Mannich bases of isatin and its derivatives with trizole. Arzneimittel Forsch 50(1): 55-59
4. K. Gangarapu, S. Manda, A. Jallapally et al., "Synthesis of thiocarbohydrazide and carbohydrazide derivatives as possible biologically active agents," Medicinal Chemistry Research, vol. 23, no. 2, pp. 1046-1056, 2014
5. Mamedova YV, Hasanova AE, Gasimova Sz, Huseynova RA, Mamedov IG. Some isatin based Synthesis. New materials, Compoundds and Application, 2020; 4(1) : 16-9.
6. Prudhomme M. Advances in Anticancer Agents in Medicinal Chemistry. [Internet]. Sharjah: Bentham Science Publishers; 2013 [cited 2021 Sep 24].
7. Singh R, Sharma S, Banarasi B. Synthesis, Characterization And Evaluation of 2-Imino Benzothiazole Derivatives As Anticonvulsant Agents. International Journal of Pharmaceutical Sciences and Research. 2021; 5(5):213-217.