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SYNTHESIS OF NOVEL N1, 6- DISUBSTITUTED 5-CYANO-2-THIOURACIL DERIVATIVES AS ANTINOCICEPTIVE AGENTS

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In an attempt to establish new therapeutic agents, a series of 5-cyano N1, 6- disubstituted, 2-thouracil derivatives were synthesized. The titled compounds (T1-T9) were screened for antinociceptive activity using acetic acid induced writhing in mice in the dose of 5 mg/kg body weight by intraperitoneal route (*i.p.*). All compounds produced a significant analgesia in tested mice when compared with control. The analgesic activity of all compounds was also comparable to that of pentazocine, which was used as a standard in the same dose. It could be concluded that compounds bearing bulkier and lipophilic substituent on phenyl group, located at C-6 of thiouracil nucleus, were more active than hydrophilic substituents on phenyl group at the same position.

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1. Introduction

Pyrimidine derivatives have always attracted the attention of medicinal chemists because of their many therapeutic applications. One of the possible reasons for the growing interest in pyrimidine heterocycle is that, it comprises the base for thiamine, uracil and cytosine nitrogen bases which are building blocks of the nucleic acids. Pyrimidines and related fused heterocyclic derivatives are of great biological interest such as anti-inflammatory / analgesic [1, 2], antimicrobial [3, 4] and antileishmanial [5]. The uracil derivatives of pyrimidine are well known for their enzyme inhibition [6-8] and antiretroviral [9-11] properties. The C_5 position of pyrimidine nucleus is an excellent target for modification or substitution [12]. However, the structure activity relationship studies revealed that, C-6 position is also an important determinant for the activity [5]. This stimulated our interest to synthesize some novel thiouracil derivatives of biological importance.

In continuation of our previous work [13], in this article the attempts have been made to synthesize and explore the antinociceptive activity of some novel N1, 6- disubstituted 5-cyano-2-thiouracil derivatives. In addition to earlier compounds some new analogues of titled nucleus were synthesized and evaluated for antinociceptive activity. The structures of the compounds were confirmed by FTIR and NMR spectroscopy studies whereas, their antinociceptive activity was evaluated by chemical nociception model of acetic acid induced writhing response in mice.

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2. Material and methods

2.1. Materials

The melting points were determined in open capillary tubes and are uncorrected. The purity of compounds was checked by TLC on silica gel G plates. IR spectra were recorded on Digilab FT-IR spectrophotometer. ^{1}HNMR spectra were recorded in DMSO (d6) and $CDCI_3$ on Varian Mercury YH-300 NMR spectrophotometer. Microwave assisted reactions were carried out in a Catalysts Microwave synthesizer.

2.2. Animals

Adult male mice (Swiss strain 22-25 g) were used for evaluation of antinociceptive activity. The animals were housed under standard environmental conditions (light period of 12 h/day, temperature 25-27°C and relative humidity 30-70%) with access to food and water *ad libitum*. The experiment was performed according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and the experimental protocol was approved by the Institutional Local Animal Ethical Committee [14].

2.3. Methods

2.3.1. Synthesis of N1, 6-diaryl substituted- 5-cyano -2- thiouracil derivatives (T1-T9)

The desired compounds (T1-T9) were synthesized by the tertiary condensation of aryl-substituted thiourea (0.01 mol), appropriate aldehyde (0.01 mol) and ethylcyanoacetate (0.01 mol) catalyzed by potassium carbonate in presence of small amount of ethanol (one- pot reaction). The reaction mixture was subjected to microwave pulse for 120 s (240 w), 60 s (350 w) and 60 s (450 w) for 4-8 minutes, to improve the percentage yield (Fig. 1). After the completion of reaction, the precipitate of potassium salt obtained, was dried, dissolved in warm water and acidified by acetic acid to precipitate pure nucleobase. The crude product was recrystallised from acetic acid. All the compounds were obtained in good yield.

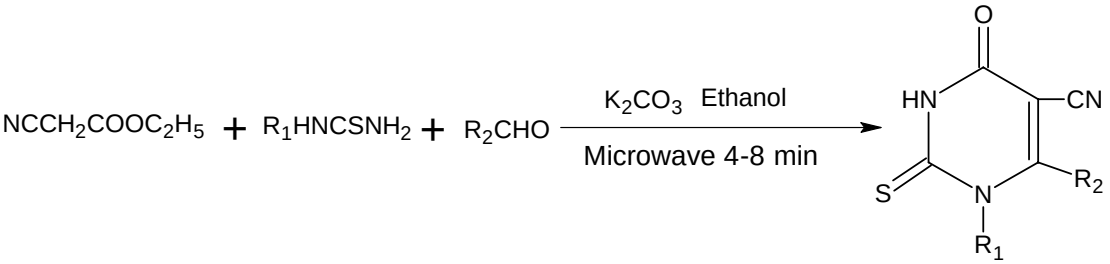

Fig.1 Scheme of synthesis of thiouracil derivatives

Previously the aryl-substituted thioureas were obtained by reacting various substituted aromatic amines with ammonium thiocyanate in presence of concentrated hydrochloric acid. The compounds were recrystallized from hot water or ethanol and used further for the synthesis of thiouracil derivatives [15, 16].

T1: 5-Cyano-1, 6-diphenyl-2-thiouracil

Yield: 72%, M.P.: 145-147°C, I.R.: ν cm^{-1} 3495 (N-H), 3005 (C-H), 2270 (C≡N), 1700 (C=O), 1605 (C=C), 1435 (C-N), 1265 (C=S), 705 (phenyl), ^{1}HNMR: δ (ppm): δ 9.28 (s, 1H, NH), δ 7.17-7.40 (m, 10H, ArH), Anal: Found: C, 66.85; H, 3.55; N, 13.75; S, 10.47. Calcd for $C_{17}H_{11}N_3SO$: C, 66.87; H, 3.63; N, 13.76; S, 10.50 %.

T2: 5-Cyano-1-phenyl-6- (4-hydroxyphenyl)-2-thiouracil

Yield: 83%, M.P.: 166-169°C, I.R.: ν cm^{-1} 3500 (O-H), 3363 (N-H), 3010 (C-H), 2210 (C≡N), 1680 (C=O), 1622 (C=C), 1452 (C-N), 1180 (C-O), 1305 (C=S), 810 (phenyl), ^{1}HNMR: δ (ppm): 8.17 (s, 1H, NH), 7.24-7.95 (m, 9H, ArH), 4.33-4.40 (s, 1H, OH), Anal: Found: C, 63.52; H, 3.39; N, 13.05; S, 9.93. Calcd for $C_{17}H_{11}N_3SO_2$: C, 63.54; H, 3.45; N, 13.08; S, 9.98%.

T3: 5-Cyano-1- (4-fluoro-phenyl)-6-(4-hydroxyphenyl)-2-thiouracil

Yield: 65%, M.P.: 168-169°C, I.R.: ν cm^{-1} 3512 (O-H), 3388 (N-H), 3014 (C-H), 2260 (C≡N), 1690 (C=O), 1610 (C=C), 1450 (C-N), 1320 (C=S), 1165 (C-F), 807 (phenyl), ^{1}HNMR: δ (ppm): 8.19 (s, 1H, NH), 7.38-7.90 (m, 8H, ArH), 4.31-4.37 (s, 1H, OH), Anal: Found: C, 60.11; H, 2.92; N, 12.33; S, 9.39. Calcd for $C_{17}H_{10}FN_3SO_2$: C, 60.17; H, 2.97; N, 12.38; S, 9.45%.

T4: 5-Cyano-1- (4-methyl-phenyl)-6-phenyl-2-thiouracil

Yield: 70%, M.P.: 140-142°C, I.R.: ν cm^{-1} 3370 (N-H), 3100 (C-H aromatic), 2995 (C-H aliphatic), 2240 (C≡N), 1678 (C=O), 1620 (C=C), 1463 (C-N), 1200 (C=S), 805 (phenyl), ^{1}HNMR: δ (ppm): 8.00-8.84 (s, 1H, NH), 7.18-7.98 (m, 9H, ArH), 2.35-2.60 (d, 3H, CH_3), Anal: Found: C, 67.69; H, 4.05; N, 13.13; S, 10.01. Calcd for $C_{18}H_{13}N_3SO$: C, 67.69; H, 4.10; N, 13.16; S, 10.04 %.

T5: 5-Cyano-1-phenyl-6 - (4-methoxy-phenyl)-2-thiouracil

Yield: 69%, M.P.: 209-211°C, I.R.: ν cm^{-1} 3410 (N-H), 3005 (C-H), 2250 (C≡N), 1665 (C=O), 1590 (C=C), 1465 (C-N), 1280 (C=S), 1275 (O-CH_3), 801 (phenyl), ^{1}HNMR: δ (ppm): 8.13 (s, 1H, NH), 7.34-7.95 (m, 9H, ArH), 3.77-3.92 (s, 3H, OCH_3), Anal: Found: C, 64.42; H, 3.88; N, 12.50; S, 9.53. Calcd for $C_{18}H_{13}N_3SO_2$: C, 64.46; H, 3.91; N, 12.53; S, 9.56 %.

T6: 5-Cyano-1- (3-methyl-phenyl)-6-(4-methoxy-phenyl)-2-thiouracil

Yield: 74%, M.P.: 275-278°C, I.R.: ν cm^{-1} 3380 (N-H), 3000 (C-H aromatic), 2990 (C-H aliphatic), 2205 (C≡N), 1680 (C=O), 1605 (C=C), 1451 (C-N), 1250 (C=S), 1170 (O-CH_3), 790 (phenyl), ^{1}HNMR: δ (ppm): 8.13 (s, 1H, NH), 7.30-7.98 (m, 8H, ArH), 3.88 (s, 3H, OCH_3), 2.17 (s, 3H, CH_3), Anal: Found: C, 65.29; H, 4.27; N, 12.01; S, 9.13. Calcd for $C_{19}H_{15}N_3SO_2$: C, 65.31; H, 4.33; N, 12.03; S, 9.18 %.

T7: 5-Cyano-1- (4-fluoro-phenyl)-6-(4-methoxy-phenyl)-2-thiouracil

Yield: 78%, M.P.: 195-197°C, I.R.: ν cm^{-1} 3377 (N-H), 3051 (C-H aromatic), 2240 (C≡N), 1685 (C=O), 1592 (C=C), 1447 (C-N), 1262 (C=S), 1280 (O-CH_3), 1135 (C-F), 802 (phenyl), ^{1}HNMR: δ (ppm): 8.13-8.15 (s, 1H, NH), 7.33-7.98 (m, 8H, ArH), 3.85-3.90 (s, 3H, OCH_3), Anal: Found: C, 61.33; H, 3.36; N, 11.90; S, 9.06. Calcd for $C_{18}H_{12}FN_3SO_2$: C, 61.18; H, 3.42; N, 11.89; S, 9.07 %.

T8: 5-Cyano-1- (4-nitro-phenyl)-6-(4-methoxy-phenyl)-2-thiouracil

Yield: 71%, M.P.: 264-266°C, I.R.: ν cm^{-1} 3332 (N-H), 3005 (C-H), 2245 (C≡N), 1675 (C=O), 1595 (C=C), 1445 (C-N), 1250 (C=S), 1162 (O-CH_3), 1395 (N-O), 795 (phenyl), ^{1}HNMR: δ (ppm): 8.02 (s, 1H, NH), 7.37-7.91 (m, 8H, ArH), 3.86-3.89 (s, 3H, OCH_3), Anal: Found: C, 67.01; H, 5.28; N, 14.86; S, 8.49. Calcd for $C_{21}H_{20}N_4SO$: C, 67.00; H, 5.35; N, 14.88; S, 8.52 %.

T9: 5-Cyano-1- (2, 4-dimethyl-phenyl)-6- (4-methoxy-phenyl)-2-thiouracil

Yield: 68%, M.P.: 253-256°C, I.R.: ν cm^{-1} 3415 (N-H), 3010 (C-H aromatic), 2993 (C-H aliphatic), 2245 (C≡N), 1680 (C=O), 1605 (C=C), 1440 (C-N), 1275 (C=S), 1175 (O-CH_3), 806 (phenyl), ^{1}HNMR: δ (ppm): 8.13-8.15 (d, 1H, NH), 7.32-7.98 (m, 7H, ArH), 2.16-2.17 (s, 6H, CH_3), 3.88 (s, 3H, OCH_3), Anal: Found: C, 66.09; H, 4.65; N, 11.53; S, 8.79. Calcd for $C_{20}H_{17}N_3SO_2$: C, 66.10; H, 4.71; N, 11.56; S, 8.82 %.

2.3.2. Evaluation of antinociceptive activity

The antinociceptive activity was evaluated using acetic acid-induced writhing (abdominal constriction test) test [17, 18]. All compounds were dissolved in DMSO: water (1:4) and injected intraperitoneally at 5 mg/kg doses in mice (n=5 in each group). A mixture of DMSO: water (1:4) was used as a control for comparison [19]. Thirty minutes after treatment, the mice were given an intraperitoneal injection of 0.6% v/v acetic acid in a volume of 10 ml/kg to induce the

characteristic writhings. The number of writhings occurring between 5 and 15 min after acetic acid injection was recorded. The response of the drug treated animals was compared with that of the animals receiving pentazocin (standard 5 mg/kg) and control. Antinociceptive activity was expressed as the percentage inhibition of writhing response between placebo control animals and mice pretreated with compounds.

3. Results and discussion

3.1. Chemistry

The synthesis of substituted N1, 6-disubstituted 5-cyano, 2-thiouracil derivatives (T1-T10) was accomplished by condensing different aromatic aldehydes, substituted thioureas and ethylcyanoacetate in presence of potassium carbonate and small amount of ethanol. Microwave irradiation of the reaction mixture at different pulse intensities and appropriate time intervals had accelerated the rate of reaction as evidenced by higher yields of the products [20]. The structures of the compounds were confirmed by spectral analysis.

All compounds were characterized by principal absorption IR bands at 2205-2270 cm^{-1}, 3332-3495 cm^{-1}, 3000-3100 cm^{-1}, 1605-1690 cm^{-1}, 1200-1320 cm^{-1} 1435-1465 cm^{-1}, and 705-810 cm^{-1}, indicating symmetric / asymmetric stretching and / or deforming vibrations caused by cyanide, amide N-H, aromatic C-H, amide carbonyl, thiocarbonyl, ring C-N and phenyl functional groups respectively. In addition to the skeletal bands of ring; compounds containing hydroxyl, fluoro, methyl, methoxy and nitro groups, produced their characteristic IR bands at 3500-3512 cm^{-1}, 1135-1165 cm^{-1}, 2990-2995 cm^{-1}, 1162-1280 cm^{-1} and 1395 cm^{-1} respectively. In support with vibrational spectroscopic analysis, ^{1}HNMR investigations revealed the appearance of singlet or in some cases doublet in the range of 8.00-9.28 (δ) assignable to NH protons of the ring. The NMR peaks at 7.17-7.98 could be assignable to aromatic protons. The signals at 4.31-4.40, 2.16-2.60 and 3.77-3.92 were exhibited by hydroxyl, methyl and methoxy protons respectively. Thus spectral data was in full agreement with the proposed structures of the compounds.

3.2. Antinociceptive activity

The present work was undertaken to assess the antinociceptive effect of the newly synthesized N1, 6 disubstituted thiouracil derivatives for their analgesic property. The analgesic activity was demonstrated on chemical nociception in the test model of acetic acid-induced writhing. It has been reported that the acetic acid-induced abdominal constriction is believed to show the involvement of peripheral mechanisms [21]. However, to investigate the involvement of central mechanisms, pentazocin was used as a standard and the activities of the compounds were also compared with pentazocin, administered in the same dose (5 mg/kg). Table 1 shows percentage inhibition of writhing reflex in the animals treated with control, standard and test compounds. It is evident that all thiouracil derivatives exhibited significant antinociceptive activity in acetic acid induced nociceptive model. The results demonstrated that all thiouracil derivatives reduced the number of abdominal writhing (43.89-79.81%) significantly and potently ($p < 0.001$) as compared to control animals (Table 1). The positive control group treated with pentazocin has shown highest inhibitory response (97.26%, $p < 0.001$). It has been reported that acetic acid induces the release of endogenous mediators, such as PGE2 (prostaglandin E2) and PGF2α in peritoneal fluids as well as lipooxygenase, indirectly, which stimulate the nociceptive neurons sensitive to analgesics [22, 23]. The antinociceptive activity of thiouracil derivatives was comparable to pentazocin as both of them have resulted in significant reduction in writhing response at an earlier phase. This indicated the possibility of the involvement of the central mechanisms along with peripheral mechanisms in the analgesic activity of thiouracil derivatives which is necessary to be confirmed using other models of nociception. However, the result of the acetic acid induced writhing response strongly suggested a partial involvement of inhibition of

lipooxygenase and/or cyclooxygenase in peripheral tissues, thereby reducing PGE2 synthesis and interfering with the mechanism of transduction in primary afferent nociceptor [24].

Table1. Antonociceptive activity of 5-cyano, N1, 6-disubstituted, 2-thiouracils in mice by acetic acid induced writhing response

Compound No. Dose: 5 mg/kg	Writhing response* n = 5	Percentage inhibition of writhing reflex
Placebo Control	41.67 ± 3.31	------
T1	17.79 ± 2.73 †	57.32
T2	23.38 ± 3.24 †	43.89
T3	09.77 ± 1.77 †	76.56
T4	11.46 ± 1.89 †	72.49
T5	08.43 ± 1.53 †	79.78
T6	14.07 ± 2.12 †	66.24
T7	08.41 ± 1.36 †	79.81
T8	09.37 ± 1.68 †	77.51
T9	08.88 ± 1.77 †	78.69
Pentazocine	1.14 ± 0.47 †	97.26

* indicates mean of five writhing responses in five mice ± S.D. n = number of animals in each group (5); † indicates *p* value compared to control $p < 0.001$. S.D.: standard deviation.

When antinociceptive activity of thiouracil derivatives was correlated with their structural features, it could be concluded that the presence of hydrophilic substituent on aromatic moiety at C-6 position resulted in decrease in the activity. However, this effect could be nullified by introduction of an electron withdrawing or bulkier group on the aromatic moiety at N1-position. In conclusion, lipophilic substituents are more favorable on aromatic moieties for an appreciable antinociceptive activity.

4. Conclusion

The present study investigated an antinociceptive activity of some newly synthesized thiouracil derivatives using acetic acid induced nociception model in mice. All compounds were found to be significantly effective as antinociceptive agents. According to the results, it could be concluded that the compounds might be acting by peripheral mechanisms via inhibition of prostaglandin synthesis. However, further studies are necessary to confirm the involvement of central mechanisms in their antinociceptive effect in tested animals.

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