ANTIPYRETIC EVALUATION OF SYNTHESIZED SEMICARBAZONE DERIVATIVES

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Summary

In the present study we have used pharmacophore hybridization technique of drug design and designed a pharmacophore model methylphenylsemicarbazone which is having hydrogen acceptor site, hydrogen donor site, lipophilic site etc using ligandscout-2.02 software. A series of methylphenylsemicarbazone was synthesized and evaluated for their antipyretic activity using Brewer's yeast induced pyrexia in rats. Based on the results of an antipyretic study, compound 25 was the most active compound.

Keywords: Pyrexia, Chalcone, Semicarbazone, Brewer's yeast, Antipyretic

Introduction

Pyrexia is caused as a secondary impact of infection, malignancy or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive[1]. Normally the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediator's (cytokines like interleukins and TNF- α), which increase the synthesis of prostaglandin E2 (PGE2) near peptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature[2]. High fever often increases faster disease progression by increasing tissue catabolism, dehydration and existing complaints, as found in HIV[3]. Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by blocking the metabolism of arachidonic acid (AA) through the enzyme cyclooxygenase (COX) and thereby the production of prostaglandins, e.g. PGE2[4]. Semicarbazone, themselves are of much interest due to a wide spectrum of pharmacological activities like antibacterial**,** antifungal[5], anticonvulsant[6], antitubercular[7] analgesic and anti-inflammatory[8] etc. There are several reports about the synthesis and pharmacological evaluation of new bioactive Naroylarylhydrazones acting at the AA cascade enzyme level and chalcones are also having analgesic and anti-inflammatory activity[9]. On these observations, we have designed a synthetic scheme to synthesize this pharmacophore, and also synthesize some lead compounds and their antipyretic activity was evaluated using brewer;s yeast induced pyrexia in rats.

Materials and methods

Methylphenyl semicarbazones were previously synthesized and characterized[10]. Melting points were measured in open capillary tubes on a Buchi 530 melting point apparatus and were uncorrected. Infrared (IR) and proton nuclear magnetic resonance (1H NMR) spectra were recorded for the compounds on Jasco IR Report 100 (KBr) and Brucker Advance (300 MHz) instruments, respectively. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. All exchangeable protons were confirmed by addition of D_2O. Mass spectra were measured with a Shimadzu GC-MS-QP5000 spectrophotometer. Only molecular ions (M+) and base peaks are given. Elemental analysis (C, H and N) were undertaken with a Perkin-Elmer model 240C analyzer, and all analyses were consistent with theoretical values (within 0.4%) unless indicated. The homogeneity of the compounds was monitored by ascending thin-layer chromatography (TLC) on silica gel G (Merck) coated aluminum plates, visualized by iodine vapor[11]. The structure (Figure 1) and physicochemical properties of the synthesized title compounds are given in Table 1.

Table 1: Physicochemical data of methylphenyl semicarbazones

Comp no.	R	R1	R2	Yield (%)	Mol Wt.	Mol Formula	Mp (^oC)	Rf Value
4	2- CH_3	H	H	57	371	$C_{23}H_{21}N_3O_2$	150	0.78
14	4-CH_3	H	H	52	371	$C_{23}H_{21}N_3O_2$	206	0.53
24	2- CH3	H	p-Cl	65	389.88	C23H20ClN3O	115	0.49
25	2-CH3	H	Cinnamaldehyde	73	381.47	C25H23N3O	126	0.51
26	2-CH3	p-NH2	p-Cl	61	404.89	C23H21ClN4O	192	0.73
27	4-CH3	p-NH2	H	63	370.45	C23H22N4O	180	0.68
28	4-CH3	p-NH2	p-Cl	63	404.89	C23H21ClN4O	173	0.72

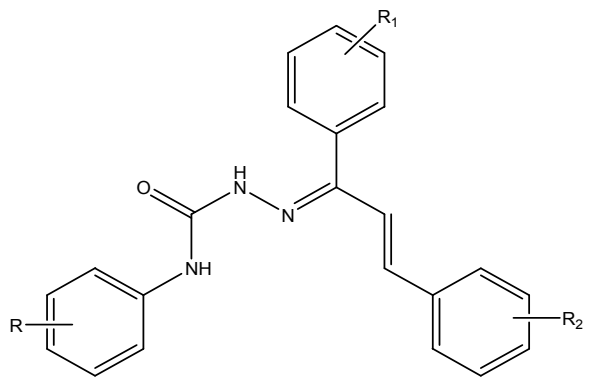

Figure 1: Structure of synthesized title compounds

Yeast induced hyperthermia in rats

The protocol for animal experimentation was approved by Institutional Animal Ethics Committee. Yeast induced pyrexia was used to evaluate the antipyretic activity of synthesized compounds. Before experimentation basal rectal temperature of rats were recorded by inserting a well lubricated bulb of a thermometer in the rectum. Rats were injected subcutaneously with 10 mL kg^{-1} body weight brewer's yeast suspension (15% in 0.5% w/v methylcellulose) to induce pyrexia. Nineteen hours after yeast injection, the rectal temperature was recorded again and animals showing a rise in temperature of $<0.6^{o}C$ were discarded. Thereafter, treatment was carried out. Test animals were orally administered 30mg/kg of the synthesized compounds, saline (10ml kg^{-1}; p.o.; control) or 100mg/kg Aspirin (Reference drug). Finally, rectal temperatures were recorded by digital thermometer at 1, 2 and 3 h intervals after administration of compounds[12].

Percentage inhibition of elevated temperature (pyrexia) was calculated using formula:

$$\% \text{ inhibition of elevated temperature (pyrexia)} = \frac{(B-A)-(C-A)}{(B-A)} \times 100,$$

Where A is norman temperature; B is temperature after 19 Hr of brewer's yeast administration and C is the temperature at different time interval after Standard/Synthesized compounds administered.

All the data were given as Mean±SD for 6 rats. $P<0.001$, 0.01 & 0.05 were considered significant. Mean difference in temperature elevation was measured statistically by One way ANOVA followed by Turkey test.

Results and discussion

The Antipyretic activity of the synthesized methylphenyl semicarbazone compounds was evaluated using Brewer's yeast induced pyrexia in rats which is summarized in Table 2.

Among the synthesized compounds, compound 25 showed the better or comparable activity in comparision to the standard drug. The lengthening of carbon chain i.e. cinnameldehyde (compound 25) increased antipyretic activity significantly. The chlorine substitution in the aldehydic moiety (compound 24, 26, 28) and amino substitution in acetophenic moiety (compound 26, 27, 28) is not favorable for the antipyretic activity although it increases the antipyretic activity of the compounds more than parent compounds but not significantly.

The compounds with no substitution (compound 4, 14) or less substitution were showed very less protection against pyrexia in comparison to the substituted compounds.

Table 2: Effect of chalcone semicarbazones on yeast induced hyperthermia in rats

Compound	Dose (mg/kg)	Rectal Temperature (^{0}C)*		Rectal Temperature after administration of compound (^{0}C)*		
		Normal (A)	19 h after yeast admin. (B)	20 h (C1)	21 h (C2)	22 h (C3)
Control	----	100.75±0.23	103.88±0.12	103.95±0.1	103.97±0.08	103.95±0.13
Aspirin	100	100.7±0.14	103.97±0.06	102.15±0.11 (55.66)[a]	101.42±0.24 (77.98)[a]	100.95±0.19 (92.35)[a]
4	30	100.8±0.14	103.97±0.16	103.3±0.18 (21.14)[d]	103.07±0.22 (28.39)[a,d]	102.95±0.13 (32.18)[a,d]
14	30	100.45±0.19	103.63±0.15	103.03±0.14 (18.87)[d]	102.78±0.12 (26.73)[b,d]	102.6±0.08 (32.39)[a,d]
24	30	100.28±0.16	103.53±0.13	102.6±0.14 (28.61)[b,e]	102.42±0.1 (34.46)[a,d]	102.23±0.13 (40)[a,d]
25	30	100.45±0.13	103.68±0.1	102.08±0.11 (49.54)[a]	101.62±0.15 (63.78)[a]	101.47±0.13 (68.42)[a,e]
26	30	100.42±0.2	103.57±0.18	102.6±0.23 (30.79)[a,f]	102.48±0.21 (34.29)[a,d]	102.32±0.18 (39.68)[a,d]
27	30	100.43±0.21	103.7±0.21	102.8±0.16 (27.52)[b,d]	102.27±0.15 (44.04)[a,d]	102.25±0.18 (44.34)[a,d]
28	30	100.42±0.21	103.58±0.14	102.65±0.12 (29.65)[a,f]	102.38±0.17 (37.85)[a,d]	102.27±0.15 (41.64)[a,d]

Figures in parenthesis indicate inhibition (%) of temperature elevation.
[a,b] $P < 0.001$ and 0.01 compared with control; [d,e,f] $P < 0.001$, 0.01 and 0.05respectively compared with standard; One way ANOVA test followed by turkey test.*Each value is the mean ±S.D. for 6 rats.

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