SYNTHESIS OF NEW BARBITURIC ACID DERIVATIVES AND THEIR ANTIVIRAL AND ANTI-INFLAMMATORY EVALUATIONS

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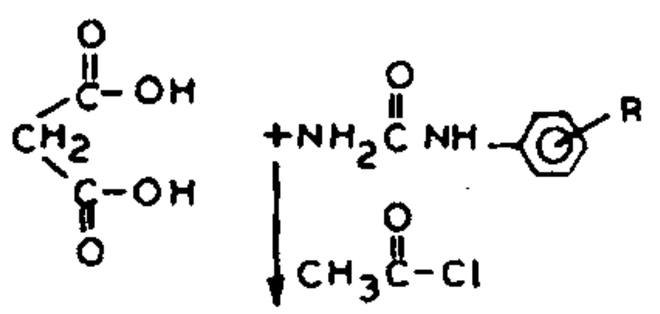
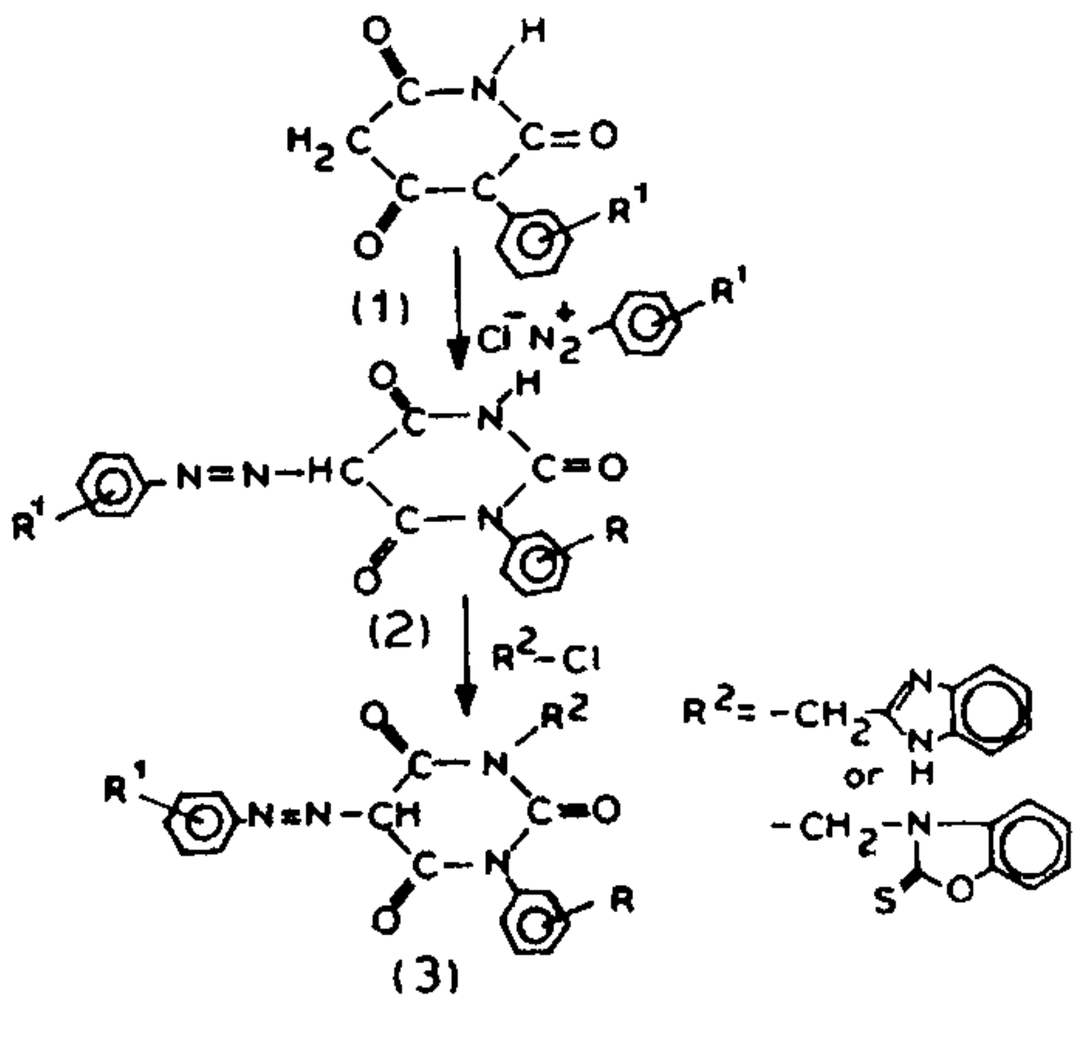
ABSTRACT

Twelve 1-aryl-5-arylazo barbituric acid derivatives (2) were synthesized by the coupling of aryl diazonium chloride on position-5 of 1-aryl barbituric acids (1) [Japp–Klinglmann reaction]. Further twenty-four 1-aryl-3-(2′-methyl benzimidazolyl/benzoxazolin-2′-thione-3′-methyl)-5-arylazo barbituric acids (3) were also prepared by the condensation of benzimidazolyl-2-methyl chloride/benzoxazolin-2′-thione-3′-methyl-chloride at position-3 of the compound (2). All the synthesized compounds were screened for their antiviral property against two plant viruses, TMV and CGMMV, both *in vivo* as well as *in vitro* and 14 compounds were also tested for their anti-inflammatory activity.

INTRODUCTION

BARBITURIC acid derivatives are well known for their wide range of biological activities[1] such as antiviral and anti-inflammatory. At the same time azo derivatives[2] benzimidazole[3,4] and benzoxazole[5] nuclei also are biologically important. It was therefore thought of interest to synthesize the title compounds by incorporating the arylazo moiety at position-5 of compound (1) and substituted benzazoles at position-5 of the compound (2) and to screen them for their antiviral and anti-inflammatory properties. Compound (1) has been prepared by the reaction of malonic acid and N-aryl substituted urea in the presence of acetyl chloride. Compound (1) undergoes the coupling reaction with aryl diazonium chloride at the active methylene group site position-5 (Japp–Klinglmann reaction) in the presence of methanol and sodium acetate to give compound (2). Compound (3) was synthesized by the condensation of compound (2) with benzimidazole-2-methylchloride/benzoxazolin-2′-thion-3′-methyl chloride in the presence of alcoholic KOH. Structural assignments of the compounds were based on IR, PMR spectra and elemental analyses Synthetic route is given in scheme 1.





Scheme-1

EXPERIMENTAL

All the melting points were determined in open capillary tubes using H_2SO_4 and are uncorrected. The structures of the compounds were confirmed by IR and PMR spectra and elemental analyses. The IR spectra were recorded on Perkin–Elmer spectrophotometer in KBr (max in cm^{-1}) and PMR spectra in $DMSOd_6$ on a Varian A-90D instrument using TMS as internal standard (chemical shift in δ). Purity of the compounds was checked on TLC.

1-Aryl substituted barbituric acids (1)

Appropriate N-arylurea (0.001 mol) and malonic acid (0.015 mol) were heated slowly on a water bath

with acetylchloride (7 ml) at 60–80° for 1 hr. After cooling the pasty mass was triturated with water and the solid separated was filtered, recrystallized from ethanol (yield 60–70%). Four compounds were thus synthesized and are given in table 1—I.R. (cm^{-1}):

3400 (NH), 2800 (CH), (C, peak as in two shoulders).

1-Aryl-5-arylazosubstituted barbituric acids (2)

Freshly prepared aryl diazonium salt solution was added dropwise to a cooled solution of compound (1) [0.01 mol] in methanol (20 ml) containing sodium acetate (500 mg) and the temperature was maintained below 5° during the addition. The reaction mixture was then kept overnight at room temperature and poured into ice-cooled water. The solid separated was filtered, washed with cold water and recrystallized from methanol (yield 65–75%). The compounds thus synthesized are given in table (2). IR (cm^{-1})

Table 1 *1-Aryl substituted barbituric acids (1)*

Compound 1	R	Molecular formula	M.P. [°C]
a	3-CH_3	$C_{11}H_{10}N_2O_3$	125
b	2-OCH_3	$C_{11}H_{10}N_2O_4$	165
c	4-CH_3	$C_{11}H_{10}N_2O_3$	170
d	2-CH_3	$C_{11}H_{10}N_2O_3$	145

Elemental analyses results were found within the satisfactory limits.

Table 2 *1-Aryl-5-arylazo substituted barbituric acids (2)*

Compound 2	R	R'	Molecular formula	M.P. [°C]
a	3-CH_3	4-Cl	$C_{17}H_{13}N_4O_3Cl$	110
b	3-CH_3	3-NO_2	$C_{17}H_{13}N_5O_5$	105
c	3-CH_3	4-CH_3	$C_{18}H_{16}N_4O_3$	115
d	2-OCH_3	4-Cl	$C_{17}H_{13}N_4O_4Cl$	157
e	2-OCH_3	3-NO_2	$C_{17}H_{13}N_5O_6$	155
f	2-OCH_3	4-CH_3	$C_{17}H_{16}N_4O_4$	152
g	4-CH_3	4-Cl	$C_{17}H_{13}N_2O_3Cl$	185
h	4-CH_3	3-NO_2	$C_{17}H_{13}N_5O_5$	180
i	4-CH_3	4-CH_3	$C_{18}H_{16}N_4O_3$	194
j	4-CH_3	4-Cl	$C_{17}H_{13}N_4O_3Cl$.	138
k	4-CH_3	3-NO_2	$C_{17}H_{13}N_5O_5$	135
l	4-CH_3	4-CH_3	$C_{18}H_{16}N_4O_3$	144

Elemental analyses results were found within the satisfactory limits.

a = 3350 (NH), 2800 (CH), 1700 (C), 1600 (N=N).

b = 3200 (NH), 2900 (CH), 1700 (C), 1600 (N=N), 1500 and 1360 (NO_2).

1 - Aryl - 5 - arylazo-3-[(benzimidazolyl-2'-methyl)/(benzoxazolin-2'-thione-3'-methl)]-substituted barbituric acids (3)

These were synthesized by refluxing an equimolar (0.01 mol) quantities of compound (2) and 2'-chloromethylbenzimidazole or benzoxazolin-2'-

Table 3 *1-Aryl-3-(2'-methylbenzimidazole benzoxazolin-2'-thione-3'-methyl)-5-arylazo substituted barbituric acids (3)*

Compound 3	R	R^1	R^2	Molecular formula	M.P. [°C]
a	3-CH_3	4-Cl	$C_8H_7N_2$	$C_{25}H_{19}N_6O_3Cl$	above 300
b	3-CH_3	4-Cl	C_8H_6NOS	$C_{25}H_{18}N_5O_4SCl$	120
c	3-CH_3	3-NO_2	$C_8H_7N_2$	$C_{25}H_{19}N_7O_5$	190
d	3-CH_3	3-NO_2	C_8H_6NOS	$C_{25}H_{18}N_6O_6S$	185
e	3-CH_3	4-CH_3	$C_8H_7N_2$	$C_{26}H_{22}N_6O_3$	210
f	3-CH_3	4-CH_3	C_8H_6NOS	$C_{26}H_{21}N_5O_4S$	260
g	2-OCH_3	4-Cl	$C_8H_7N_2$	$C_{25}H_{19}N_6O_4Cl$	997
h	2-OCH_3	4-Cl	C_8H_6NOS	$C_{25}H_{18}N_5O_5SCl$	88
i	2-OCH_3	3-NO_2	$C_8H_7N_2$	$C_{25}H_{19}N_7O_6$	100
j	2-OCH_3	3-NO_2	C_8H_6NOS	$C_{25}H_{18}N_6O_7S$	120
k	2-OCH_3	3-NO_2	$C_8H_7N_2$	$C_{26}H_{22}N_6O_4$	110
l	2-OCH_3	3-NO_2	C_8H_6NOS	$C_{26}H_{21}N_5O_5S$	120
m	4-CH	4-Cl	$C_8H_7N_2$	$C_{25}H_{19}N_6O_3Cl$	154
n	4-CH	4-Cl	C_8H_6NOS	$C_{25}H_{18}N_5O_4SCl$	145
o	4-CH	3-NO_2	$C_8H_7N_2$	$C_{25}H_{19}N_7O_5$	134
p	4-CH_3	3-NO_2	C_8H_6NOS	$C_{25}H_{18}N_6O_6S$	105
q	4-CH_3	4-CH_3	$C_8H_7N_2$	$C_{26}H_{22}N_6O_3$	210
r	4-CH_3	4-CH_3	C_8H_6NCS	$C_{26}H_{21}N_5O_4S$	160
s	2-CH_3	4-Cl	$C_8H_7N_2$	$C_{25}H_{19}N_6O_3Cl$	above 300
t	2-CH_3	4-Cl	C_8H_6NOS	$C_{25}H_{18}N_5O_4SCl$	above 300
u	2-CH_3	3-NO_3	$C_8H_7N_2$	$C_{25}H_{19}N_7O_5$	285
v	2-CH_3	3-NO_3	C_8H_6NCS	$C_{25}H_{18}N_6O_6S$	above 300
w	2-CH_3	4-CH_3	$C_8H_7N_2$	$C_{26}H_{22}N_6O_3$	220
x	2-CH_3	4-CH_3	C_8H_6NOS	$C_{26}H_{21}N_5O_4S$	above 320

Elemental analyses results were found within the satisfactory limits.

thione-3′-methyl chloride in 50 ml of 5% alcoholic KOH for 12–14 hr on a steam bath. The excess solvent was distilled and the reaction mixture was poured over crushed ice. The excess of KOH was neutralized with acetic acid. The solid separated was filtered and recrystallized from methanol water (yield 50–55%). Compounds thus synthesized are given in table 3.

IR (cm^{-1}) a = 3200 (NH), 2900 (CH), 1680 (C=O), 1600 (N=N). o = 3400 (NH), 2900 (CH), 1680 (C=O), 1600 (N=N), 1560 and 1360 (NO_2), p = 2900 (CH), 1680 (C=O), 1600 (N=N), 1540 and 1360 (NO_2), 1200 (C=S)

PMR(δ) c = 2–1.3 (3H, CH_3, singlet), 3.2 (2H, CH_2, singlet) 6.7–6.6 (H, CH singlet), 7–7.3 (12H, aromatic H, hump) 8.5 (1H, NH benzimidazole moiety, singlet).

Chloromethyl-2′-benzimidazole was prepared according to an earlier reported method[6]. 2-Thione-3′-chloromethylbenzoxazole was also prepared according to a reported method[7].

Antiviral activity against plant virus: All the compounds listed in tables 1, 2 and 3 were tested for their antiviral activity against TMV and CGMMV both *in vivo* as well as *in vitro* using *D. stramonium* and *C. amaranticolor* as host plants respectively by following the reported method[8].

The virus cultures were maintained on their respective hypersensitive hosts in the plant virus laboratory of the Botany Department, Lucknow University. The virus inoculum was prepared by grinding 1 g of fresh leaves showing severe disease symptoms in a sterilized mortar and pestle with an equal amount (w/v) of distilled water. The pulp was squeezed through two-folds of muslin cloth and the juice was centrifuged at 3000 g for 15 min. The supernatant liquid was diluted to 1 : 100 in distilled water and used as inoculum.

The solutions of the unknown compounds were prepared by dissolving 10 mg of the compound in

Table 4 *Effect of compound 1, 2 and 3 on Tobacco mosaic virus (TMV) and Cucumber green mottle mosaic virus (CGMMV)*

Compound	Inhibition [%] *in vivo*	Against TMV *in vitro*	Inhibition [%] *in vivo*	Against CGMMV *in vitro*	Compound	Inhibition [%] *in vivo*	Against TMV *in vitro*	Inhibition [%] *in vivo*	Against CGMMV *in vitro*
1a	47	75	52	63	3e	52	67	67	72
1b	40	43	38	53	3f	28	48	24	47
1c	66	71	48	53	3g	40	46	32	43
1d	12	08	13	16	3h	47	52	31	48
2a	37	38	30	32	3i	37	40	18	32
2b	—	—	—	—	3j	22	39	27	30
2c	—	—	—	—	3k	50	58	34	52
2d	—	—	—	—	3l	60	73	67	70
2e	22	34	21	32	3m	46	53	59	61
2f	18	26	19	23	3n	NS	NS	NS	NS
2g	66	71	36	62	3o	52	55	40	44
2h	63	69	31	52	3p	08	18	04	16
2i	50	57	46	50	3q	12	21	26	37
2j	20	24	26	27	3r	—	—	—	—
2k	21	34	30	37	3s	28	31	30	36
2l	22	26	20	28	3t	40	48	36	43
3a	62	80	62	76	3u	37	49	37	50
3b	62	69	61	74	3v	31	60	49	57
3c	68	82	61	73	3w	50	57	32	59
3d	37	43	32	42	3x	34	63	50	58

— = Nil; NS = Not screened.

1 ml of ethanol and total volume was made up to 10 ml by adding distilled water. Solutions were rubbed on the upper surface of leaves of the test plant 24 hr prior to the virus challenge. The leaves of control plants were rubbed with distilled water. In the case of *in vitro* experiments the appropriate virus and compounds were mixed (1 : 1) before inoculation, incubated for 30 min at room temperature and applied on the leaves of the test plants. Controls consisted of leaves rubbed with virus in which distilled H_2O was added instead of compounds. Carborandum powder (600 mesh) was used as an abrasive.

All the experiments were performed in an insect-free glass house at about 20–30°C. Local lesions were counted 5–6 days after virus inoculated and the percentage inhibition was calculated by the formula $[(C-T)/C] \times 100$, where C is the number of local lesions on control and T on the treated leaves. The data were analyzed statistically[9].

All the compounds exhibited activity of varying degree against both the viruses compounds no. 1c, 2g, 2h, 3a, 3b, 3c, 3e and 3l caused significant inhibition against TMV (50–60%) *in vivo*, 70–82% *in vitro*. Compound 3c had maximum inhibition of 68% *in vivo* and 82% *in vitro*, while compound no. 3a, 3b, 3c, 3e, 3l and 3m showed good activity against CGMMV (59–67% *in vivo*, 61–76% *in vitro*). Compound 3l caused maximum inhibition of 67% *in vivo* in this case. All the compounds were given at a dose level of 1 mg/ml and results are recorded in table 1.

From SAR point of view, one can infer that all the compounds having an electron-releasing group at *p*-position of the benzene ring at position-1 of barbituric acid (R=4-CH_3) inhibited the viral growth significantly. Further the presence of chlorine at *p*-position of arylazo moiety retained the activity of the parent compound (1) while presence of a nitro group at *m*-position of the same moiety somewhat decreased the activity. It was also observed that the incorporation of benzimidazole and benzoxazole moieties at position-3 of the compound (2) increased the activity in some compounds (3a, 3b, 3c, 3e and 3l). It can also be mentioned that a benzimidazole moiety made the compound more antiviral in comparison to benzoxazolin-2-thione.

Anti-inflammatory activity

Compounds were screened for their anti-inflammatory activity by carrageenin induced rat paw oedema test according to the procedure of Winter *et al*[10]. ALD_{50} values of all the tested compounds were evaluated by a reported method[11].

The activity was done in groups of five mice weighing between 20 and 25 g. The inflammatory oedema was induced by carrageenin (4 mg/kg) and it was given to all mice except one group which was treated as control for comparison of the activity of the compounds with healthy animals. The right paw of the mice was cut and weighed 1 hr prior to the carrageenin administration. The synthetic compound was administered at a dose level of 68 mg/kg or 100 mg/kg and after 2 hr of the drug administration, the right paw was cut and reduction in the weight of the paw of mice from that of the control mice was marked as the inhibition of inflammation. For comparison of the activity a known drug butazolidine (30 mg/kg) was given to one group of mice, instead of the synthetic compounds.

Fourteen compounds (1b, 2a, 2e, ef, 2g, sj, 3d, 3k, 3l, 3o, 3s and sw) were screened for their anti-inflammatory action at two different dose levels 68 mg/kg of 100 mg/kg. Only one compound 2a showed 19% inhibition at a dose level of 100 mg/kg while control (butazolin) showed 26% inhibition at a dose level of 30 mg/kg. ALD_{50} values of these compounds indicate their nontoxic nature.

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ANNOUNCEMENTS

COMPUTER COMMUNICATIONS FOR DEVELOPING COUNTRIES CONFERENCE—1987

CCDC '87 (Computer Communication for Developing Countries), a Conference of special interest and relevance to the developing world, is being organized at Vigyan Bhavan, New Delhi from October 27–30, 1987. It is being sponsored by the International Council for Computer Communication, an organization committed to foster scientific research, development and applications of computer communications. It is being hosted by the Department of Electronics, Government of India.

The theme of the Conference will be 'Computer Communication and Issues of Relevance to Developing Countries'. Topics to be covered would include Computer and satellite communications, Office information systems, Message systems, Teletext and videotex, ISDN evolution, Fibre optics, Network design, Management and applications, Characteristics of information society for Developing world, Distributed processing, Operational experiences, Problems and challenges in introduction of new technologies and training and education. Eminent specialists from all over the world will be speaking at the Conference. Four hundred delegates —200 from India and 200 from abroad— are expected to participate in the Conference.

Enquiries by those who wish to participate or contribute papers to this Conference are welcome to contact Dr S. Ramani, Chairman, Programme Committee, CCDC '87, National Centre for Software Technology, Gulmohar Cross Road, No. 9, Juhu, Bombay 400 049.

INDIAN ASSOCIATION FOR RADIATION PROTECTION—DR A. K. GANGULY FELICITATION PRIZE–1987

The Indian Association for Radiation Protection (IARP) has instituted a biennial prize for the best work done by any Indian Scientist in the field of Radiation Protection/Radiation in the Environment. This Award is instituted on the basis of an Endowment given by the students of Dr A. K. Ganguly to felicitate him.

Nominations are invited for the Heads of Institutions/guiding teachers/immediate superiors/ colleagues of any Indian Scientist who had, in their opinion, done exceptional work in the field of Radiation Protection/Radiation in the Environment. The work should have been carried out in India during the period January 1981 to December 1985.

Nominations should contain the following details: 1. A brief biodata of the nominee; 2. A short write-up (not exceeding 500 words) highlighting the major contributions of the person nominated; 3. A list of relevant publications in the field of work by the nominee (multi-authored papers, of which the nominee is a major contributor also may be included); and 4. Name, address and signature of the person nominating.

The prize consists of a citation and a cash award of Rs. 1,000 which will be awarded during the forthcoming XIV Conference of IARP to be held at Bombay during January 19–22, 1987.

The nominations may please be sent to: Dr T. S. Iyengar, Convenor, IARP Programmes, Health Physics Division, Bhabha Atomic Research Centre, Bombay 400 085.