Physical Sciences Section

Pak. j. sci. ind. res., vol.39, nos.1-4, Januay-April 1996

SYNTHESIS AND CARDIOVASCULAR ACTIVITY OF NEW PHENOTHIAZINES

ASHOK KUMAR , R. S. VERMA, S. K. BHATIA, SWATI GUPTA, U.K. JETTLY*, S. K. BAJPAI**

K. K. SAXENA AND V. K. SRIVASTAVA

Department of Pharmacology and Pharmacy, L. L. R. M. Medical College, Meerut-250004 (U.P.), India

(Received May 29, 1993)

Some new Schiff's bases substituted-benzaldehyde, 2-(2-acetyl-10H-phenothiazin-10-yl)-2-oxo-ethyl hydrazones (IVa-IVe), 2-acetyl-10-(5-substituted phenyl-2-oxo-4-thiazolidin-1-yl)-aminoacetylphenothiazines (Va-Ve), 2-acetyl-10-[(2-oxo-3-chloro-4-azetidin-1-yl) aminoacetylphenothiazines (VIa-VIe) and 1, 3-disubstituted phenyl-5-[(2-acetyl-10-phenothiazin-10-yl)-2-oxo-ethyl] formazans (VIIa-VIIb) have been synthesized and were evaluated for their cardiovascular activity. Compounds IVa, IVd and IVe showed highly statistically significant changes in blood pressure, heart rate and pressor responses (Cardiovascular activity). The most active compound in our preliminary study is found to be 2, 4-dichlorobenzaldehyde 2-(2-acetyl-10H-phenothiazin-10-yl)-2-oxo-ethyl hydrazone (IVa) which showed statistically significant changes in the fall of blood pressure (75.63%).

Key words: Phenothiazine derivatives, Cardiovascular activity, Toxicity studies.

Introduction

Phenothiazines have been reported to possess anti-psychotic [1], anti-parkinsonian [2], anti-inflammatory [3] and cardiovascular activities [4,5]. Furthermore, thiazolidinones, azetidinones and formazans of various pharmacodynamic heterocyclic nuclei have also been found to possess a wide spectrum of biological properties, viz. anti-parkinsonian [6], anti-inflammatory [7,8] and cardiovascular [9,10]. In the light of these observations we have synthesized Schiff's bases (IVa-IVe), thiazolidinones (Va-Ve), azetidinone (VIa-VIe) and formazans (VIIa-VIIe) of 2-acetyl-phenothiazine. The structure of the compounds were elucidated by IR, ^{1}H-NMR and mass spectrometry. The compounds were also screened for cardiovascular activity.

Materials and Methods

Synthesis of drugs. Analytical data of C, H and N were within ± 0.4% of the theoreitical values. Melting points were taken in open capillary tube and are uncorrected. IR spectra were recorded on a Perkin-Elmer 157 spectrometer in KBr pellet (υ_{max} in cm^{-1}). The purity of the compounds was checked by TLC, ^{1}H-NMR spectra were recorded on a Em-360 spectrometer using TMS as internal reference. The mass spectra were recorded on a JMD-300 double focussing spectrometer fitted with a JMA-2000. Physical and analytical data of the compounds are given in Table 1. The required 2-acetylphenothiazine (I) [11], 2-acetyl-10-(chloroacetyl) phenothiazine (II) [5] and 2-acetyl-10-(hydrazinoacetyl) phenothiazine (III) [5] are prepared by already known methods in literature.

(a). Substituted-benzaldehyde-2-[(2-acetyl-10-H-phenothiazin-10-yl)-2-oxo-ethyl] hydrazones (IVa-IVe). To a solution of 2-acetyl-10-(hydrazinoacetyl) phenothiazine (III) (0.01 mol) in toluene (dry, 50 ml) and a few drops of glacial acetic acid was added to the respective substituted benzaldehyde (0.01 mol) and the mixture was refluxed for 16-22 hrs. The solvent was distilled off to yield sticky residue which was washed with petroleum ether (40-60°C) and recrystallized from appropriate solvents. IR of IVa showed bands at 1640, 1710 and 3400 cm^{-1}, characteristic of C=N, C=O and NH stretching respectively. ^{1}H-NMR ($CDCl_3$; IVa)δ 6.85-8.35 (m, 10H, ArH), 8.60 (s, 1H, -N=CH-), 4.45 (hump, 1H, CH_2NH, exchangeable), 2.55 (d, 2H, J=9.00Hz, CH_2NH), 2.30 (s, 3H, OCH_3) ppm. $[M]^+$ m/z 470 (Molecular ion peak).

(b). 2-Acetyl-10-[(5-substituted-phenyl-2-oxo-4-thiazolidin-1-yl)-aminoacetyl]-phenothiazines (Va-Ve). To a cold solution of the hydrazones (0.01 mol) was added mercaptoacetic acid (0.02 mol) dropwise with stirring at ambient temperature and the mixture was kept for 6 days at room temperature and refluxed for 10 hrs. It was then filtered, concentrated, cooled and poured into ice water. The resulting solid was recrystallized from appropriate solvent. IR of Va showed bands at 1680 cm^{-1}, characteristic of C=O with an additional band at 1740 cm^{-1} of γ-thialactam ring. ^{1}H-NMR (TFA): δ 7.30-8.60 (m,10H, ArH), 8.65 (s, 1H,-N-CH), 2.35 (s, 3H-$COCH_3$) 2.50 (d, 2H, 9J=Hz, CH_2NH) 4.50 (s,1H, CH_2NH, exchangeable), 5.75 (s, 2H, CH_2 of

*Department of Chemistry, D. N. College Meerut (U.P.) Pin.250004, India.

**Department of Postportum Programme, L. L. R. M. Midical College, Meerut Pin 250004 (U.P.), India.

thiazolidin-4-one ring) ppm. $[M]^+$ m/z 544 (Molecular ion peak).

(c). 2-Acetyl-10-[(2-oxo-3-chloro-4-azetidin-1-yl) aminoacetyl] phenothiazines (VIa-VIe) [6]. To a solution of hydrazones (IVa-IVe; 0.01 mol) and triethylamine (0.02 mol) in dry DMF (50 ml), chloracetylchloride (0.02 mol) was added dropwise during 1 hr. with stirring and the reaction mixture was kept at room temperature overnight followed by refluxing for 6-10 hrs. The resulting solid was filtered off, concentrated and poured into crushed ice. The solid thus separated was recrystallized from appropriate solvents. IR of VIa showed bands at 3410 (NH), 1700 (C=O) and 1760 (C=O) of β-lactam ring). ^{1}H-NMR ($CDCl_3$): δ 6.40-8.10 (m,10H, ArH), 5.45 (d, 1H, J=4.5 Hz, -CH-Ar), 4.65 (d, 1H, J=4.5 Hz, CHCl), 4.45 (hump, 1H, NH, CH_2NH, exchangable), 2.60 (d, 2H, J=4.5Hz, CH_2NH), 2.30 (s, 3H, $COCH_3$), ppm. $[M]^+$ m/z 546 (molecular ion peak).

(d). 1,3-Disubstituted-phenyl-5-[(2-acetyl-10H-phenothiazin-10-yl)-2-oxo-ethyl] formazans (VIIa-VIIe) [13]. To the substituted anilines (2g) dissolved in glacial acetic acid (5 ml) was added concentrated HCl (3 ml) at 0-5°C. A solution of $NaNO_2$ (1g in 5 ml of water) was then added dropwise. The diazonium salt solution thus prepared to IVa dropwise with stirring in pyridine (50 ml). The reaction mixture was kept at room temperature for 2-3 days and then poure into cold water (250 ml). The resulting solid was washed with water and recrystallized from appropriate solvents. IR of VIIa showed bands at 3050 cm^{-1} of aromatic C-H, 1720 cm^{-1} CO, 1640 cm^{-1} of C=N, 1420 cm^{-1} of N=N. ^{1}H-NMR ($DMSOd_6$; VIIa) δ 7.85 -9.95 (m, 14H, Ar-H), 2.36 (s, 3H, $-COCH_3$) 5.60 (s. 1H, $-CH_2NH$, exchangeable), 5.70 (d, 1H, J=9Hz, CH_2NH), 2.25 (s, CH_3-Ar) ppm. $[M]^+$ m/z 588 (Molecular ion peak).

Animal studies. Dogs (20-25 kg of either) were anaesthetized with α-chloralose (80 mg/kg i.v.) and maintained on positive artificial respiration to avoid cardiovascular changes secondary to changes in respiratory activity. The animals were divided into two groups of 5 animals each. One of the group was treated as control group while another group was given drugs intravenously (from femoral vein) by dissolving them in propylene glycol and the effect on blood pressure, heart rate and pressor responses evoked either by carotid occulsion (CO) or intravenous noradrenaline 1-2 μg/kg iv injection (NA). 0.25 ml of propylene glycol was injected as vehicle to see the effect of the vehicle on the above parameters. Appropriate lethal dose (ALD_{50}) of the promising compounds was determined in albino mice of either sex weighing 20-30 grams according to the method [13]. The results (Table-2) were analysed by using paired student 't' test.

Results and Discussion

Injection of 0.25 ml of propylene glycol induced only transient decrease of 5 to 10 mmHg in blood pressure with no changes in CO and NA responses.

The compounds of this series (all the four stages, see scheme) showed hypotensive activity of varying degree (5-90 mmHg) and duration (6-190 mins).

The compound IVa which was substituted with chloro group at 2 and 4 positions of phenyl ring, when administered at a dose of 5 mg/kg i.v. elicited biphasic response. The initial rise in blood pressure (140±5.91 mmHg) was followed by potent and gradual fall in blood pressure (29±7.49) as compared to control value. The hypotensive activity of this compound lasted for 190±6.32 mins and was accompanied with decrease in heart rate (83±1.16 beats/mins) as compared to control value (92±1.22 beats/mins). The percentage fall in blood pressure was 75.63. In addition, it was associated with abolition of CO and without affecting NA responses, which is suggestive of central site of action of the compound. Moreover, this compound showed highly significant values (>0.001) of all the parameters for cardiovascular system.

In view of its potentialities it was studied in three graded doses (1.25, 2.50 and 5.0 mg/kg i. v). In the lowest dose it did not showed statistically significant activity. However, this compound, at a dose of 2.5 mg/kg exhibited biphasic response, initial rise in blood pressure (112±3.39 mmHg) followed by potent and gradual fall (70±2.23 mmHg). The hypotensive activity of this compound lasted for 46±1.87 mins and was associated with decrease in heart rate (87±1.76 beats/min.) as compared to control (93±1.99 beats/min.). The hypotensive activity of this compound was associated with partial inhibition of CO and without affecting the NA pressor responses. The percentage fall in blood pressure at this dose is less (30). This compound has shown statistically significant values in all the parameters on cardiovascular system. The compound IVb which was substituted by chloro group at 3 position of phenyl ring showed hypotensive activity but it was not found to be statistically significant. On the contrary, compound IVc which was substituted with chlorogroup on 4 position, possessed highly significant value for blood pressure (52±62 mmHg) while change in heart rate is not significant.

When phenyl ring was substituted with highly electronegative fluoro group at 2-position (IVd) showed highly significant but less potent hypotensive activity (percentage fall in blood pressure 63.82) than compound IVa. The hypotensive activity of this compound IVd was accompained with inhibition or abolition of CO response without affecting NA response, which is an indicative of peripheral site of action of compound.

The compound IVe which was substituted at 2-position by methoxy group showed biphasic response and highly significant changes in blood pressure and heart rate but is less potent than IVa and IVd. Moreover, its activity was associated with blockade of both the pressor responses, suggesting the peripheral site of action of the compound.

The conversion of Schiff's bases (IVa-IVe) into their thiazolidinone phenothiazine (Va-Ve) (five membered ring) decrease the hypotensive activity except in Va, Ve and Vd, which has shown signficant (>0.001) hypotensive activity.

TABLE 1. PHYSICAL AND ANALYTICAL DATA OF COMPOUND IV, V, VI AND VII.

Compd No.	R	R'	M.P.°C	Yield %	Purified solvent*	molecular formula	Analysis C, H, N (%) Calcd	Found	Calcd	Found	Calcd	Found
IVa	2,4-Cl_2	-	191	50	A	$C_{23}H_{17}N_3O_2SCl_2$	58.72	58.30	3.61	3.67	8.93	8.10
IV	3-Cl	-	163	65	A/B	$C_{23}H_{18}N_3O_2SCl$	63.37	63.41	4.13	4.23	9.64	9.57
IVc	4-Cl	-	178	55	C	$C_{23}H_{18}N_3O_2SCl$	63.37	63.51	4.13	4.16	9.64	9.67
IVd	2-F	-	235	40	D	$C_{23}H_{18}N_3O_2SF$	65.87	65.63	4.33	4.35	10.02	10.13
IVe	2-OCH_3	-	187	35	A	$C_{24}H_{21}N_3O_3S$	66.82	66.57	4.87	4.91	9.74	9.81
Va	2,4-Cl_2	-	213	55	A	$C_{25}H_{19}N_3O_3S_2Cl_2$	55.14	55.53	3.49	4.41	7.72	7.83
Vb	3-Cl	-	243	70	D/E	$C_{25}H_{20}N_3O_3S_2Cl$	58.88	58.93	3.92	3.71	8.24	8.31
Vc	4-Cl	-	205	50	A	$C_{25}H_{20}N_3O_3S_2Cl$	58.88	58.69	3.92	3.99	8.24	8.41
Vd	2-F	-	195	55	B	$C_{25}H_{20}N_3O_3S_2F$	60.85	60.83	4.05	4.09	8.51	8.55
Ve	2-OCH_3	-	202	40	A/B	$C_{26}H_{23}N_3O_4S_2$	61.78	61.81	4.55	4.61	8.31	8.47
VIa	2,4-Cl_2	-	136	45	A	$C_{25}H_{18}N_3O_3SCl_3$	54.89	54.65	3.29	3.19	7.68	7.54
VIb	3-Cl	-	156	60	A	$C_{25}H_{19}N_3O_3SCl_2$	58.59	58.57	3.71	3.51	8.20	7.99
VIc	4-Cl	-	185	45	D/E	$C_{25}H_{19}N_3O_3SCl_2$	58.59	58.69	3.71	3.56	8.20	7.87
VId	2-F	-	218	30	A/B	$C_{25}H_{19}N_3O_3SCl\,F$	60.54	60.41	3.83	3.80	8.48	8.43
VIe	2-OCH_3	-	186	50	C	$C_{26}H_{22}N_3O_4SC$	61.48	61.43	4.33	4.36	8.28	8.25
VIIa	2,4-Cl_2	2-CH_3	236	50	A/B	$C_{30}H_{23}N_5O_2SCl_2$	61.22	61.32	3.91	3.94	11.90	11.93
VIIb	3-Cl	4-Cl	277	60	A	$C_{29}H_{21}N_5O_2SCl_2$	60.62	60.71	3.65	3.68	12.19	12.93
VIIc	4-Cl	3-Cl	239	55	C	$C_{29}H_{21}N_5O_2SCl_2$	60.62	60.64	3.65	3.71	12.19	12.34
VIId	2-F	2,4-Cl_2	248	70	D	$C_{29}H_{20}N_5O_2SCl_2F$	58.78	58.83	3.37	3.41	11.82	11.87
VIIe	2-OCH_3	2-CH_3	215	65	D/F	$C_{31}H_{27}N_5O_3S$	67.76	67.73	4.92	4.87	12.75	12.71

A=Ethanol, B=Water, C=Methanol, D=Benzene, E=Potroleum ether. F=Ethyl acetate.

TABLE 2. CARDIOVASCULAR ACTIVITY OF COMPOUNDS IV, V, VI, AND VII.

Compd. No.	R	R'	Dose mg/kg i.v.	Control value of b.p. mean ± SE	Change in resting Bloodpressure mm/Hg Immediate Mean ± SE	Delayed Mean ± SE	% change in BP	Duration in Minutes Mean ± SE	Control value of H.R. Mean ± SE	Change in resting H.R. bpm Mean ± SE	%change in H.R.	Effect on Pressor Responses CO	NA
IVa	2,4-Cl_2	-	1.25	92 ± 12.60	-	78 ± 5.34	-15.22	14 ± 1.43	94 ± 1.86	92 ± 2.02	-02.13	-	-
	-	-	2.50	100±07.90	112±3.39	70±2.23*	-30.00	46±1.87	93±1.99	87±1.76	-06.45	Inhibited	Unaffected
	-	-	5.00	119±16.73	140±5.91	29±7.47*	-75.63	190±6.32	92±1.22	83±1.16*	-09.78	Blocked	Unaffected
IVb	3-Cl	-	5.00	100±06.11	-	70±4.73	-30.00	17±0.97	92±2.54	89±2.86	-03.26	-	-
IVc	4-Cl	-	5.00	97±04.63	-	52±4.62*	-46.39	11±1.02	92±1.99	87±2.52	-05.43	-	-
IVd	2-F	-	5.00	94±05.33	-	34±5.78*	-63.82	76±3.67	94±0.99	79±2.91*	-15.96	Blocked	Unaffected
IVe	2-OCH_3	-	5.00	101±06.77	117±5.82	56±6.19*	-44.55	44±1.87	90±1.58	76±1.87*	-15.56	Blocked	Blocked
Va	2,4-Cl_2	-	5.00	96±05.33	-	51±4.84*	-46.87	32±1.12	92±3.39	89±3.20	-03.26	Inhibited	Blocked
Vb	3-Cl	-	5.00	99±07.13	-	84±7.30	-15.15	32±2.55	-	-	-	-	-
Vc	4-Cl	-	5.00	98±06.03	-	68±6.80*	-30.61	54±1.05	-	-	-	-	-
Vd	2-F	-	5.00	106±09.40	-	31±8.40*	-70.75	32±1.67	-	-	-	-	-
Ve	2-OCH_3	-	5.00	95±06.57	-	70±7.40	-06.66	44±1.85	92±3.39	89±3.05*	-03.26	Inhibited	Potentiated
VIa	2,4-Cl_2	-	5.00	95±07.06	-	69±5.98	-27.36	10±0.75	-	-	-	-	-
VIb	3-Cl	-	5.00	101±07.47	-	57±8.14	-43.56	17±2.55	-	-	-	-	-
VIc	4-Cl	-	5.00	91±05.09	-	66±5.78	-27.47	32±2.55	-	-	-	-	-
VId	2-F	-	5.00	97±08.87	-	57±8.73	-41.23	42±2.55	-	-	-	-	-
VIe	2-OCH_3	-	5.00	95±03.53	-	65±1.58*	-31.57	30±3.53	-	-	-	-	-
VIIa	2,4-Cl_2	2-CH_3	5.00	102±06.43	-	92±5.60	-09.80	10±1.16	-	-	-	-	-
VIIb	3-Cl	4-Cl	5.00	107±10.66	-	102±9.93	-04.67	06±1.02	-	-	-	-	-
VIIc	4-Cl	3-Cl	5.00	104±05.33	-	99±5.29	-04.81	06±0.58	-	-	-	-	-
VIId	2-F	2,4-Cl_2	5.00	101±10.28	-	86±9.65	-14.85	15±0.99	-	-	-	-	-
VIIe	2-OCH_3	2-CH_3	5.00	95±07.59	-	80±6.31	-15.79	18±2.55	-	-	-	-	-

* p>0.001; ** ALD50 of Compound (IVa-IVe) and (Va-Ve) was > 1000 mg/kg i.p.

The percentage changes in the fall of blood pressure were 46.87, 30.61 and 70.61 respectively. The percentage change in compound IVd was comparable to IVa at a dose of 5 mg/kg i.v. but this compound did not show any effect on heart rate and pressor responses, therefore suggestive of direct vasodilator.

The conversion of IVa-IVe into their corresponding azetidinone phenothiazines (four membered ring) (VIa-VIe) showed mild to moderate activity since these compounds did not elicit any effect on the pressor responses and heart rate except compound VIe showed statistically significant change (65±1.58) in blood pressure.

The conversion of Schiff's bases into their corresponding formazanphenothiazines (VIIa-VIIe) exhibited hypotensive activity of mild degree (5-15 mmHg) and duration (6-18 mins) which was not significant statistically (>0.05).

Interestingly enough, the compounds of four stages have shown diffrent pharmacological profiles, clonidine like centrally acting as compound IVa, secondly a purely peripheral beta adrenergic blockade agent compound IVe and directly acting vasodilator compounds.

Toxicity studies. ALD50 of the promising compound was found to be >1000mg/kg i.p. suggesting a good safety margin.

References

1. R. J. Baldessarini, *The Pharmacological Basis of Therapeutics* (Goodman and Gilman's, Pergamon Press, Maxwell Macmillan, Internal, 1992), Vol. I, 8th ed., pp.386-401.
2. P. Kumar, C. Nath, J. C. Agarwal, K.P. Bhargava and K. Shanker, Indian J. Chem., **22B,** 952 (1933).
3. M. Verma, V. R. Gujrati, A. K. Saxena and K. Shanker, J. Indian Chem. Soc., **58** (11), 1119 (1981).
4. J. Gante, H. E. Radunz, Orth. Dieter. Schlip. Hans. Jochen. Schorscher Ernst (Merck Patent G.m.b.H.), Ger. Offen, 2, 917, 650 (Cl.CO7D 417/06), 13 Nov. 1980, Appl. 02 May (1979).
5. A. Kumar, S. Gurtu, J. C. Agarwal, J. N. Sinha, K. P. Bhargava and K. Shanker, J. Indian Chem. Soc., **60,** 608 (1983).
6. P. Kumar, C. Nath, K. P. Bhargava and K. Shanker, Indian J. Chem., **21B,** 1128. (1982).
7. Atul Kumar, A. Kumar, A. K. Saxena and K. Shanker, Pharmazie, **43** (11.1), 45 (1988).
8. I. P. Singh, S. Gurtu, A. Kumar, J. N. Sinha, K. P. Bhargava and K. Shanker, Archiv. der Pharmazie, **317,** 904 (1984).
9. Ashok Kumar, A. K. Saxena, S. Gurtu, J. N. Sinha and K. Shanker, Indian Drugs, **24** (1), 1 (1986).
10. A. Kumar, K. K. Saxena, V. K. Srivastava, S. Lata and R. S. Saxena, J. Indian Chem. Soc., **68,** 139 (1991).
11. M. Siska, S. Lasslo and C. Lander, Acta Pharm (Hung), **32,** 770 (1910).
12. M. Tandon, Piyush. Kumar, P. Tandon, T. N. Bhalla and J. P. Barthwal, Indian Drugs, **21** (1), 24 (1983).
13. Q. E. Smith, Pharmacological Screening Test. *Progress in Medical Chemistry I* (Butterworth, London, 1960).