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Synthesis, Biological and Pharmacological Activities of Some New Derivatives of 5-Aryl-1,3,4-oxadiazolin-2-thiones

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Four different S-(5-aryl-1,3,4-oxadiazol-2-yl)mercaptoacetic acids were prepared from the respective 5-aryl-1,3,4-oxadiazol-2-thiones on reaction with chloroacetic acid. Each of these acids has been converted into its acid chloride using thionyl chloride and condensed with three different secondary amines: dicyclohexylamine, morpholine and piperidine. The products obtained in each case were purified and characterized as the respective N,N-disubstituted S-(5-aryl-1,3,4-oxadiazol-2-yl) mercaptoacetamides. Alternatively, acid chlorides were also reacted *in situ* with two different N-substituted 2-aminoethanols and the products were characterized as the respective 2-N,N-substituted aminoethyl S-(5-aryl-1,3,4-oxadiazol-2-yl) mercaptoacetates. Similarly the acid chlorides *in situ* were also substituted to a reaction with 2-methoxyethanol and 2-ethoxyethanol, the products were purified and characterized as the respective esters, *i.e*., 2-alkoxyethyl S-(5-aryl-1,3,4-oxadiazol-2-yl) mercaptoacetates. On the basis of their analytical and spectral data, the new derivatives of oxadiazol-2-thiones were evaluated for their antimicrobial (antibacterial and anti fungal) activities, by standard methods and found to exhibit relatively good antibacterial activity specifically against *P. auroginosa* and antifungal activity against *C. lunata*. The fenamates in which the carboxylic group replaced by oxadiazolin-2-thione were reported to exhibit antiinflammatory activity by inhibiting the cyclo-oxygenase and 5-lipoxygenase activities. Ibuprofen, a known NSAID was structurally modified by a similar replacement of its carboxylic group with oxadizolin-2-thione and was to be effective at an oral dose of 100 ug/kg (bw). Some oxadiazolyl trizoles were reported to exhibit invitro antimicrobial properties. A series of quinazolinonyloxadiazoles were synthesized and found to show good oral hypoglycemic activity. Some of the N-mannich bases of β-(N-substituted indolyl)-1,3,4-oxadiozolin-2- thiones were synthesized and found to exhibit anti-inflamatory activity. A similar compound with an acetic acid hydrazino group at 3-position were found to show a broad spectrum antibacterial activity. Herein, the synthesis, characterization, biological and pharmacological activities of some S-substituted analogues of 5-substituted 1,3,4 -oxadiazolin-2-thiones are reported.

Key Words: 5-Aryl-1,3,4-oxadizolin-2-thiones, 2-Aminoethanols, Antimicrobial activity, Anticholololinergic activity, Antihistaminic activity.

INTRODUCTION

The fenamates in which the carboxylic group replaced by oxadiazolin-2-thiones were reported to exhibit antiinflammatory activity by inhibiting the cyclo-oxygenase and 5-lipoxygenase activities[1]. Ibuprofen, a known NSAID was structurally modified by a similar replacement of its carboxylic group with oxadiazolin-2-thione and was

to be effective at an oral dose of 100 g/kg (bw)[2] some oxadiazolyl triazoles were reported to exhibit *in vitro* antimicrobial properties[3]. A series of quinazolinon-yl-oxadiazoles were synthesized and found to show good oral hypoglycemic activity[4]. Some of the N-Mannich bases of 3-(N-substituted)aminomethyl-3-(2-indolyl)-1,3,4-oxadiazolin-2-thiones were synthesized and found to exhibit antiinflammatory activity[5]. A similar compounds with an acetic acid hydrazine group at 3-position were found to show a broad spectrum of antibacterial activity[6].

These results have prompted us to synthesize some S-substituted analogues of 5-substituted-1,3,4-oxadiazolin-2-thiones and characterized and also to screen them for their possible antimicrobial, analgesic, antiinflammatory and ^{1}H-antihistaminic activities by standard methods. Keeping in view of the structural features of such compounds against those reported to possess such activities.

EXPERIMENTAL

Four different 5-aryl-1,3,4-oxadizol-2-thiones, were prepared from the respective aromatic acid hydrazides by known methods and identified. The reactions were monitered by TLC.

Melting points of the compounds were determined in open capillaries using Cintex melting point apparatus and are uncorrected. The IR spectra were recorded on Perkin-Elmer Infracord-283 spectrophotometer, as KBr pellets, H NMR spectra on OMEGA-500 MHz spectrometer using TMS as an internal standard and mass spectra on a FENNIGAN MAT-90 in the EI mode and by the direct inlet method.

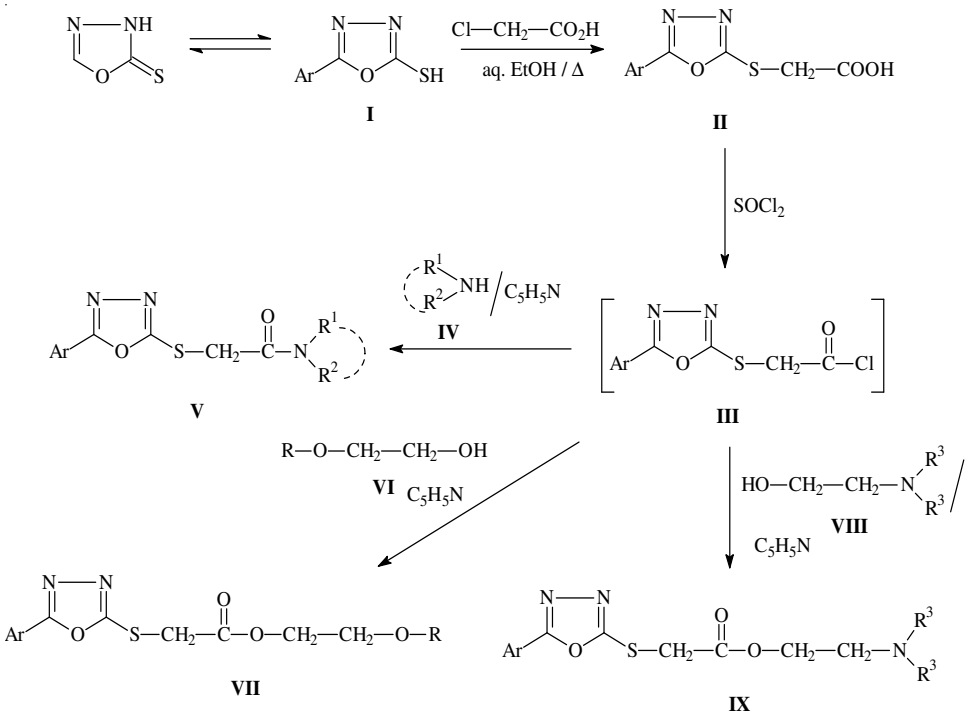
Synthesis of S-(5-aryl-1,3,4-oxadizol-2-yl) mercaptoacetic acids: II. General procedures: To a solution of 5-aryl-1,3,4-oxadiozolin-2-thiones (**I**, 0.01 mL) in alcoholic sodium hydroxide (4 %; 10 mL), chloroacetic acid (0.012 mol) was added and heated under reflux for 4 h. The reaction mixture was cooled, filtered, if necessary neutralized with dilute hydrochloric acid. The resultant product was filtered, washed with small portion of cold water and dried. It was purified by recrystalization from appropriate solvents adopting the above specified procedure (Table-1, **Scheme-I**). The following four different oxadizole mercaptoacetic acids were prepared and characterized.

Synthesis of N,N-disubstituted S-(5-aryl-1,3,4-oxadiazol-2-yl)mercapto-acetamides (V): An appropriate (5-aryl-1,3,4-oxadiazol-2-yl) mercaptoacetic acid (**II**, 0.01 mol) was taken into a dry flask and thionyl chloride (0.015 mol) was added. The reaction mixture was heated under reflux for 0.5 h, on a hot water-bath, under dry conditions, using calcium chloride guard tube. After the fumes were ceased off, an appropriate secondary amine (**IV** 0.012 mol) and pyridine (2 mL) were added and heated under reflux for *ca.* 4-5 h. The reaction mixture was cooled and poured onto crushed ice (100 g) while stirring with a glass rod. The solid thus separated was filtered, washed with small portions of cold water, dried and purified by recrystallization above suitable solvent.

TABLE-1
CHARACTERIZATION DATA OF (5-ARYL-1,3,4-OXADIAZOL-2-YL) MERCAPTO ACETIC ACIDS

Compound	Ar	m.p. (ºC)	Elemental analysis (%): Found (calcd.)		
			C	H	N
IIa	C_6H_5	164	50.80 (50.84)	3.30 (3.38)	11.80 (11.86)
IIb	4-NO_2-C_6H_4	208	44.90 (44.94)	2.60 (2.62)	10.45 (10.48)
IIc	4-CH_3-C_6H_4	110	52.75 (52.80)	3.95 (4.00)	11.00 (11.20)
IId	2-Cl-C_6H_4	170	44.40 (44.44)	2.54 (2.59)	10.32 (10.37)

Purification of compounds has been effected by recrystallization from appropriate solvents *viz.*; % Yield: 72-85 %; Characterization data of a representative compound: **IId**: IR: in cm^{-1} at 3518-3060 (broad, OH), 725 (C=O, carboxylic), 165 (C=N, oxadiazole), ^{1}H MMR (DMSO-d_6, 8 δ pm), 3.28 (s, 2H, -S-CH_2-CO), 7.01 to 7.89 (m, 4H, Ar-H).



Ar = C_6H_5, C_6H_4—NO_2—4
C_6H_4—CH_3—4 and C_6H_4—Cl—2

R = Me, Et
(VI)

= dicyclohexylamino, 4-morpholino or 1-piperidino

(IV)

= R^3 = Me, or Et

(VIII)

Scheme-I

Synthesis of 2-(2-alkoxyethyl)-S-(5-aryl-1,3,4-oxadiazol-2-yl) mercapto-acetates (VII): To the acid chloride (*in situ*) prepared as above, added an appropriate 2-alkoxyethanol (**VI**; 0.012 mol) and pyridine (2 mL). The reaction mixture was heated under reflux for *ca.* 5-6 h. It was cooled and poured onto crushed ice (100 g) while stirring. The resultant product was filtered, washed with small portions of cold water and dried. It was purified by recrystallization from ethanol.

Synthesis of 2-(2-N, N-dialkylamino) ethyl-S-(5-aryl-1,3,4-oxadiazol-2-yl) mercaptoacetamtes (IX): An appropriate 2-dialkylaminoethanol (**VIII**; 0.012 mol) and pyridine (2 mL) were added to the acid chloride (**III**; 0.01 mol) *in situ* and heated under reflux for 5-6 h. It was then cooled and poured onto crushed ice (100 g) while stirring. The product was filtered under suction, washed thoroughly with cold water and dried. It was purified by recrystallization from the physical and analytical data of the compound **V**, **VII** and **IX** and presented in Table-2.

Biological and pharmacological assays

Antimicrobial assay: The new series of 5-aryl-1,3,4-oxadiazolin-2-thione derivatives (**V**, **VII** and **IX**) were assayed for their antimicrobial properties by standard methods.

Antibacterial activity: The antibacterial activity of all the 28 new compounds assayed against 5 different strains of bacteria *viz.* group (+)ve: *B. subtilis*, *B. mycoides*, group (-)ve: *E. coli*, *P. aeruginosa* and *P. vulgaris* bacteria by the standard agar diffusion cup-plate method[7]. The test organisms were sub-cultured using nutrient agar medium. Solutions of the test compounds were prepared by dissolving 10 mg of each in DMF (10 mL). The sterilized nutrient agar plates were inoculated with the respective strain of bacteria and made three discs of 6 mm each in each of such plates with a sterilized borer.

Then 0.1 mL (1000 ug/mL conc.) of the solution was added to the cups, aseptically and labeled, properly. Such treated plates were left undisturbed at roam temperature for at least 2 h to permit complete diffusion into nutrient agar medium. These plates were incubated at 37 ± 1 ºC for 24 h, the discs were, measured with antibiotic zone reader. Till the experiments were conducted in triplicate, controls and standards were run, simultaneously. Benzyl penicillin for group (+)ve bacteria and streptomysin for group (-)ve bacteria, each at concentration of 100 µg/mL, were employed as standards zones of substituted for standards. Benzyl penicillin: *B. subtilis* (30), *B. mycoides* (31), Streptomycin: *E. coli* (40), *P. aeruginosa* (42), *P. vulgaris* (43).

Antifungal activity: All the three series of test compounds were also assayed for their antifungal activity against two fungi: *F. oxysporum* and *C. lunata*, by standated method[7]. The test organizes were sun-cultuned using potato-dextrose-agar ueclium the tubes containing sterilized metdium were inoculated with test organisms, individually and inculcated at 25 ºC for 4 h.

TABLE-2
CHARACTERIZATION DATA OF DERIVATIVES OF (5-ARYL-1,3,4-OXADIAZOLIN-2-THIONES)MERCAPTO ACETIC ACIDS

Compound	Nature of substituted		m.p. (ºC)	Elemental analysis (%): Found (calcd.)		
	Ar	NR^1R^2/R^3		C	H	N
Va	C_6H_5	Dicyclohexylamino	265	66.14 (66.16)	7.25 (7.28)	18.98 (19.00)
Vb	C_6H_5	Morpholino	160	55.06 (55.08)	4.89 (4.91)	13.74 (13.77)
Vc	C_6H_5	Piperidino	154	59.38 (59.40)	5.59 (5.61)	13.84 (13.86)
Vd	4-NO_2-C_6H_4	Dicyclohexylamino	153	59.41 (59.45)	6.28 (6.30)	12.58 (12.61)
Ve	4-NO_2-C_6H_4	Morpholino	112	47.58 (48.00)	3.98 (4.00)	15.98 (16.00)
Vf	4-NO_2-C_6H_4	Piperidino	132	51.70 (51.72)	4.55 (4.59)	16.01 (16.09)
Vg	4-CH_3-C_6H_4	Dicyclohexylamino	180	70.90 (70.95)	7.90 (7.96)	10.75 (10.79)
Vh	4-CH_3-C_6H_4	Morpholino	100	57.80 (57.87)	5.40 (5.46)	13.45 (13.50)
Vi	4-CH_3-C_6H_4	Piperidino	162	60.50 (60.56)	5.95 (5.99)	13.20 (13.24)
Vj	2-Cl-C_6H_4	Dicyclohexylamino	167	60.91 (60.96)	6.40 (6.46)	09.65 (09.69)
Vk	2-Cl-C_6H_4	Morpholino	123	49.50 (49.55)	4.08 (4.12)	12.34 (12.38)
Vl	2-Cl-C_6H_4	Piperidino	151	56.00 (56.07)	4.94 (4.98)	13.02 (13.08)
VIIa	C_6H_5	Ethyl	134	54.50 (54.54)	5.10 (5.19)	09.05 (09.09)
VIIb	C_6H_5	Methyl	198	53.00 (53.06)	4.70 (4.76)	09.50 (09.52)
VIIc	4-NO_2-C_6H_4	Ethyl	150	47.50 (47.59)	4.20 (4.24)	11.80 (11.89)
VIId	4-NO_2-C_6H_4	Methyl	180	48.98 (46.01)	3.80 (3.83)	12.30 (12.38)
VIIe	4-CH_3-C_6H_4	Ethyl	110	55.85 (55.90)	5.54 (5.59)	08.65 (08.69)
VIIf	4-CH_3-C_6H_4	Methyl	85	54.50 (54.54)	5.12 (5.19)	09.04 (09.09)
VIIg	2-Cl-C_6H_4	Ethyl	170	49.08 (49.12)	4.35 (4.38)	08.12 (08.18)
VIIh	2-Cl-C_6H_4	Methyl	158	47.50 (47.56)	3.92 (3.96)	08.50 (08.53)
IXa	C_6H_5	Diethyl amino	160	57.25 (57.31)	6.20 (6.26)	12.50 (12.53)
IXb	C_6H_5	Dimethyl amino	145	54.69 (54.72)	5.50 (5.53)	13.60 (13.68)
IXc	4-NO_2-C_6H_4	Diethyl amino	120	50.50 (50.52)	5.20 (5.26)	14.70 (14.73)
IXd	4-NO_2-C_6H_4	Dimethyl amino	111	47.70 (47.72)	4.50 (4.54)	15.80 (15.90)
IXe	4-CH_3-C_6H_4	Diethyl amino	95	63.50 (63.55)	7.12 (7.16)	13.04 (13.08)
IXf	4-CH_3-C_6H_4	Dimethyl amino	120	51.52 (51.57)	5.40 (5.44)	12.00 (12.03)
IXg	2-Cl-C_6H_4	Diethyl amino	196	52.00 (52.02)	5.40 (5.42)	11.32 (11.38)
IXh	2-Cl-C_6H_4	Dimethyl amino	184	49.22 (49.26)	4.65 (4.69)	12.28 (12.31)

Percentage yield: **V**: 55-85 %, **VII**: 52-70 %, **IX**: 65-75 %.

Solutions of the test compounds prepared as given under antibacterial activity and placed, in two different concentrations 50-100 mg/mL, in the discs of inoculated plates. The treated plates were incubated at 20 ± 1 ºC for 48 h. Simultaneously, controls and standard were run and all experiments were conducted in triplicate. Diameters of zones of inhibition were read using antibiotic zone reader. Clotrimazole (1 mg/mL conch) was used as the standards.

Pharmacological screening: Two series of new 1,3,4-oxadiazole derivatives (**VII** and **IX**) were evaluated for their H_1-anti histaminic and anticholinergic activities.

H_1-Antihistaminic activity: The H_1-antihistaminic activity of 2-alkoxyethyl 1,3,4-oxadiazol-2yl mereapto acetates (**VII**) and 2-(N,N-alkylamino) ethyl 1,3,4-oxadiazol-2yl mereapto acetates (**IX**) was assayed by the isolated guinea pig ileum method[8]. A few centimeters long isolated guinea pig ileum was cut and placed immediately in a petridish containaing the Tyrode's solution. A 2 cm piece of such ileum was taken and tied with a thread to the top and bottom ends with at occluding the lumen and mounted in the organ-bath containing the Tyrode's solution. Temperature of the bath was maintained at 37 ± 1 ºC and was aerated, continuously. A tension of 0.5 g was applied and the tissue was allowed to equibrate for 0.5 h, before starting the experiment.

The response of the tissue by increasing doses of histamine was recorded in a smoked drum using the frontal writing lever with a magnification of 1:10. The submacimal dose of the against was selected and the response of the tissue to this dose in the presence of increasing concentrations (logarithemic doses) of the test compounds was recorded. The duration of action of the against (Histamine) on this tissue was 1 min. Each of the test compounds as its 0.8 % Na CMC solution was introduced into the organ bath and a blank was run. The solution of the test compound was allowed to act on ileum tissue for 2 min before the agonist, histamine was introduced into the organ bath.

Contractions included by the submaximal dose of the agonist in presence of the test compounds were recorded and the blockade if any produced by them was noted. Each time the tissue was allowed to rest for 3 min and effect of the agonist was ensured before the next dose was attempted. The blocking effect against logarithemic doses of each test compound was plotted and IC_{50} values were determined statistically. Avil was employed as the standard for reference.

Anticholinergic activity: Anticholinergic activity of compounds **VII** and **IX** was assayed by using the rat intestine method. Atropinc was used as an agonist to induce contractions.

The test compounds and agonist were used in logarithemic doses. A similar procedure described under antihistaminic activity was followed. The blocking effect on contractions by the test compounds against their logarithemic doses was plotted and their ± IC_{50} values were calculated, stastically.

RESULTS AND DISCUSSION

Antibacterial activity: Persual of Table-3 revealed that oxadiazol-2-yl mercaptoncetamides (**VII**) showed a varied degree of antibacterial activity, but showed activity against *P. aeruginosa*. Among all compound containing a dicyclohexylamino and 4-nitrophenyl groups was found to eahibit potent antibterial activity while the compound with morpholino group failed to act against *B. mycoides*.

Among the alkoxyethyl oxadiazol-2-yl mercaptoacetates, the compound with a methyl and phenyl substituents (**VIIa**) failed to show any antibacterial activity gainst *B. mgcoides* and *E. coli* whereas the compound with ethyl and 4-nitrophenyl.

Antifungal activity: Results of antifungal assay of oxadiazol-2-yl mercaptoacetamides (**IX**) indicate that the compound piperidino and phenyl groups failed to exhibit activity against *F. oxysporum*. The compound with dicyclohexylamino and 4-nitro phenyl groups could exhibit a mudorate antifungal activity against *C. lunata* whereas the compound with morpholino in group was more potent.

TABLE-3
DATA ON ANTIMICROBIAL ACTIVITY OF NEW
5-ARYL-1,3,4-OXADIAZOLIN-2-THIONE DERIVATIVES

Comp.	Antibacterial Activity			Antifungal Activity			
	B. subtilis	*B. mycoides*	*E. coli*	*P. aeruginosa*	*P. vulgaris*	*F. oxysporum*	*C. lunata*
Va	10	7	9	18	15	11	21
Vb	7	4	11	23	15	12	31
Vc	16	6	–	32	10	–	30
Vd	16	7	10	35	14	12	37
Ve	14	–	11	31	14	13	31
Vf	10	7	4	20	11	16	30
Vg	11	7	5	18	11	17	37
Vh	8	8	10	20	14	18	38
Vi	4	6	5	28	15	15	37
Vj	10	4	9	27	15	11	35
Vk	8	5	10	25	14	–	33
Vl	8	4	8	24	14	17	35
VIIa	14	6	8	24	20	15	36
VIIb	15	–	–	25	11	18	34
VIIc	16	–	10	32	21	14	24
VIId	10	8	10	33	10	–	27
VIIe	17	6	8	28	21	18	38
VIIf	13	6	6	30	21	14	37
VIIg	14	11	11	24	24	18	38
VIIh	17	7	–	24	25	16	28
IXa	16	8	7	24	19	–	35
IXb	16	7	10	23	16	4	27
IXc	14	–	10	37	19	5	34
IXd	16	5	9	38	15	11	35
IXe	12	7	10	32	18	15	31
IXf	11	6	8	32	17	17	33
IXg	13	5	5	26	16	16	35
IXh	13	5	4	26	15	14	33

Zone of inhibition measured in mm.

Alkoxyethyl oxadiazol-2-yl mercaptoacetates in general were shown to exhibit activity against with the organism. Compound with the methyl and 4-nitrophenyl groups was proved to exhibit good activity against *C. lunata*. While failing totally in the case of *F. oxysporum*.

Table-3 shows that all the alkoxyethyl oxdiazol-2-yl mercaptoacetates were effective against the test fungi. The compound with N,N-diethylamino and phenyl groups failed to show activity against *F. oxysporum*. The compound with diethylamino 2-chlorophenyl groups was found to be relatively more effective against *C. lunata* antihistaminic and anticholinegic.

Antichistaminic activity: Results from Table-4 reveal that the antihistaminic activity of the test compounds was not comparable to that of the standard. But when it is compared among them, the acrivity was better with 2-alkoxy ethyl (**VII**) derivatives when compared with that of 2-(N,N-dialkylamino) ethyl (**IX**) compounds. Amongst the 2-alkoxyethyl compound (**VII**), the one with ethyl (R = Et) group was superior over its methyl (R = Me) counter past.

Anticholinergic activity: Perusal of Table-4 indicates that all the eight test compounds could exhibit a variable degree of anticholinergic activity. Uniquely amongst both the series, the compounds with a 4-nitrophenyl group were found to be superior in their anticholinergic action. When the activity is compared between these two series of compounds (**VII** and **IX**) the 2-alkoxyethyl compounds (**VII**) were more potent the 2-(N,N-dialkylamino) ethyl (**IX**) compounds. Atropine at concentration of 1 mg/mL could cause contractions, effectively.

TABLE-4
DATA ON ANTIHISTAMINIC AND ANTICHOLINERGIC ACTIVITIES OF 2-ALKOXYETHYL-1,3,4-OXADIAOZOL-2YL MERCAPTOACETATES (**VII**) AND 2(N,N-DIALKYLAMINO) ETHYL 1,3,4-OXADIAZOL-2YL MERCAPTOACETAES (**IX**)

Compound	Nature of Substitutes		Antihistaminic IC_{50} (μg/mL)	Anticholinergic IC_{50} (μg/mL)
	-Ar	-R/-N(-R^1/R^2)		
VIIa	C_6H_5	-Me	829.08	212.58
VIIb	C_6H_5	-Et	771.10	223.21
VIIc	$C_6H_4.NO_2$-4	-Me	774.33	123.94
VIId	$C_6H_4.NO_2$-4	-Et	743.62	147.49
IXa	C_6H_5	-NMe_2	936.48	285.01
IXb	C_6H_5	-NEt_2	730.05	186.56
IXc	$C_6H_4.NO_2$-4	-NMe_2	958.80	142.04
IXd	$C_6H_4.NO_2$-4	-NEt_2	1151.00	148.02
	Avil (standard for H_1 antihistaminic)		480.000	–
	Atropine (standard for anticholinergic)		–	30.00

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