Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(12):162-167

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis and pharmacological study of 4-(2-oxopropylidene)-1,5 benzodiazepin-2-one and its alkylated derivatives

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ABSTRACT

The synthesis and investigation of psychotropic activity of some derivatives of 4-(2-oxopropylidene)-1,5-benzodiazepine-2-one are reported. The alkylation reactions were conducted under the conditions of phase transfer catalysis. Interestingly, the acute toxicity according to the European protocol in two mammal species appropriate to assess the lethal doses was evaluated. In addition, we studied the effects of these products on the central nervous system through a battery of behavioral tests used in psychopharmacology. The results obtained show a lack of toxicity at therapeutic doses. The products exert sedative effects at different degrees with respect to the same reference, clobazam, and lengthen the hypnotic effect of Tranxene

Keywords: Synthesis, alkylation, 1,5-benzodiazepine**,** toxicity, pharmacological activity.

INTRODUCTION

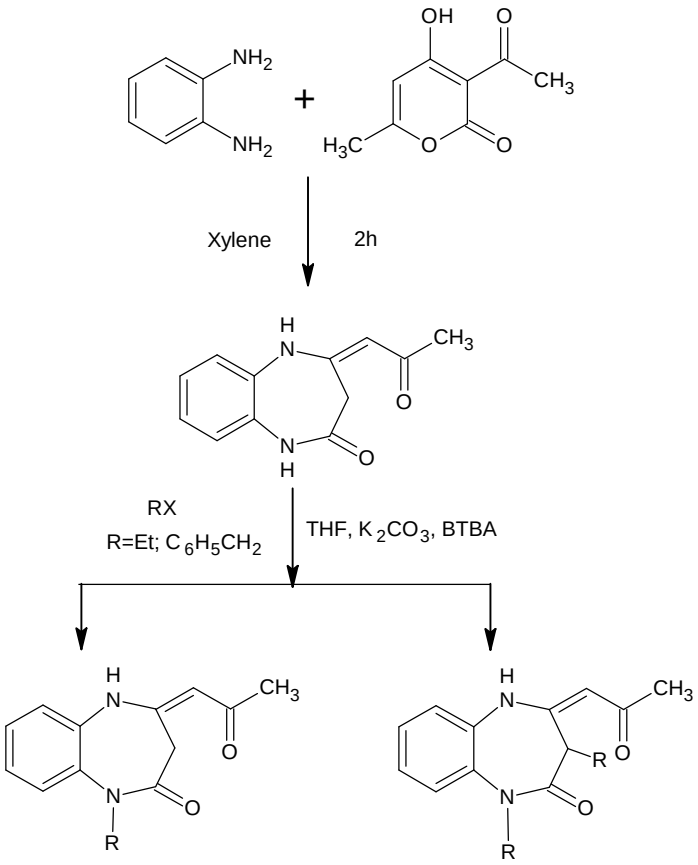
Benzodiazepines are a homogeneous both by their chemical structure and its pharmacological profile made in therapy group [1-3]. However, it has been shown that the 1.5-benzodiapezines are not devoid of activity. In particular, Urbanyl (patented by Roussel Uclaf) has been shown to have better therapeutic properties than Valium or Seresta [3].

The purpose of this paper is to synthesize alkyl derivatives of 4-(2-oxopropylidene)- 1,5-benzodiazepin-2-one and to study their effects on the central nervous system.

EXPERIMENTAL SECTION

Products

The condensation of o-phenylenediamine with dehydroacetic acid in xylene at reflux for two hours, leads to 4-(2-oxopropylidene)-1.5-benzodiazepin-2-one [4]. The product obtained was subjected to alkylation reactions under the conditions of the transfer catalysis liquid-solid phase at room temperature in THF in the presence of potassium bicarbonate and of tetrna-butylammonium bromide (T.B.A.B)as catalyst [5-6] to yield 1-ethyl-4-acetonylidene-1,5-benzodiazepine-2-one,1-benzyl-4-(2-oxopropylidene)-1,5-benzodiazepin-2-one and 1,3-dibenzyl-4-(2-oxo propylidene)-1.5-benzodiazepine-2-one as shown in Scheme 1.



Scheme 1 Scheme 1

Melting points were determined by a Tottoli device (Bushi) and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a Burker AC 250 spectrometer. Mass spectra were performed with a VARIANT MAT 311A. IR spectra were acquired by a Perkin Elmer 1760X spectrometer.

Synthesis:

Synthesis of 4-(2-oxopropylidene)-1.5-benzodiazepine-2-one

Amixture of 0.06 mole of *O*-phenylenediamine and 0.12 mol of dehydroacetic acid in xylene (80 mL) is heated at reflux for two hours with azeotropic distillation of the water formed. After evaporating the solvent under reduced pressure, the crude product obtained was chromatographed on column of silica gel (Eluant: Hexane/ ethyl acetate).

Alkylation of 4-(2-oxopropylidene)-1.5-benzodiazepine-2-one

To a solution of 0.01 mol of 4-acetonylidene 1,5-benzodiazepine-2-one in THF (60 mL) was added 0.02 mole of the alkylating agent, 0.02 mol of K_2CO_3 and 0.1 mmol of TBAB. The reaction mixture was stirred at room temperature for 48 h. After filtration, THF was removed under reduced pressure and the residue obtained was chromatographed on a silica gel column Eluent: Hexane/ ethyl acetate)..

The structures were identified on the basis of the spectral data: ^{1}H and ^{13}C NMR, IR, mass spectrometry, and the physicochemical parameters which are grouped in Table 1.

Table 1: Synthesis and spectral data of 4-acetonylidene-1,5-benzodiazepin-2-one prepared

Product	Yield (%)	Melting point	Spectral data
4-(2 oxopropyylidene-1,5-benzodiazepin-2-one	70	230-232°C (EtOH)	^{1}H NMR (DMSO-d_6, δ_{ppm}): 2,11(s, 3H, CH_3); 3,09(s, 2H, CH_2); ,27(s, 1H, CH); 7,14(m, Ar). ^{13}C NMR (DMSO-d_6) δ_{ppm}: 196,48(CO°; 167,66(CO); 154,78(C_2) 25,13-122,39(4=CH_2) MS : m/Z=216. IR : $\nu_{C=O}$ =1610 cm^{-1} , $\nu_{C=O}$=1570 cm^{-1}.
4-(2-oxopropyylidene -1-éthyl-1,5-benzodiazépin-2-one	80	110-112°C (Toulene).	MS: m/z: 244. ^{1}H NMR ($CDCl_3$,δ_{ppm}): (3,10, 2H, (s), 5,25 (1H,S), 2,10 (3H,S) X-ray:
4-(2-oxopropyylidene -1-benzyl-1,5 benzodiazepin- 2-one	60	165-167°C (Toulene).	^{1}H NMR ($CDCl_3$,δ_{ppm}):: 2,14 (S,3H,CH_3); 3,13(d,1H,CH_2); 5,35(S,1H,CH); ^{13}C NMR ($CDCl_3$,δ_{ppm}): 29,5 (CH_3), 41,4 (CH_2); 52,1 (CH_2); 96,5 (CH); 123,2-136,9 (7CHar); 132,0(Cq) MS : m/z =306
4-(2-oxopropyylidene -1,3-dibenzyl-1,5-benzodiazepin-2-one	25	145-147°C (Toulene).	^{1}H NMR ($CDCl_3$,δ_{ppm}): 2,2(s,3H,CH_3); 5,4 (s,H,CH); 3,4 (t,1H,CH); 4,7 (d,2H,CH_2) ^{13}C NMR ($CDCl_3$,δ_{ppm}):: 29,8 (CH_3); 31,8 (CH_2); 52,7 (CH_2); 47,4 (CH); 93,0 (CH)

Pharmacological Study

1-Products Tested

* 4-(2-oxopropylidene)-1,5- benzodiazepin -2- one **1**
* 1-ethyl -4 - (2-oxopropylidene) -1,5- benzodiazepin -2 - one **2**
* 1-benzyl-4-(2-oxopropylidene)-1,5-benzodiazepin -2 one **3**
*1,3-dibenzyl-4-(2-oxopropylidene)-1,5-benzodiazepin -2 one **4**

2- Reference Product:

Urbanyl® (Clobazam); 7-chloro-1-mehyl-5-phenyl-1,5-benzodiazepin-2,4-dione

3-Animals:

The experiment was conducted on Swiss mice and adult Wistar rats, in equal number, from the animal house of the Laboratory of Pharmacology, of the Faculty of Medicine and Pharmacy Rabat, Moroco. The weight of mice was between 20 to 30 g; those of rats from 100 to 200 g. Animals were housed at four per cage, allowed free access to water and food, and maintained under constant temperature (25±2 °C) and humidity (70±10%). [7,8] The use of animals was done in accordance with the manuals Animal Laboratory [9].

Method

a- Study of acute toxicity:

Five groups of 10 animals are used for determining the lethal dose of 50 LD_{50} Litchfield and Wilcoxon method [10]. Increasing doses (in geometrical progression $D_n = R^{n-1}D_1$ are administered in a volume of 0.5 mL per 20 g body weight of mouse, and 1mL per 100 g body weight of rats [11-12]. Doses of products 1-4 administered to mice were 300, 600, 900, 1200 and 2000 mg /kg and those administered to rats were 300, 600, 900, 1200.2000 mg /kg. The products are administered intraperitoneally (ip) as a suspension in a solution of 10% arabic gum. After drug administration, the animals were observed for 14 days, during which we noted the apparent clinical signs after administration of the product, the changes in body weight and mortality rates.

b- Study of psychotropic activity:

To study the sedative, anticonvulsant and hypnotic effects appealed to the following behavioral tested:

1 - Testing the traction

This test involves suspending mice by the front legs to a wire stretched horizontally. A normal mouse performs a restoration in less than 5 seconds [13].

2 - Test the chimney

The animal was placed in a test tube upside-down. A normal mouse makes the first attempt to lift the specimen within seconds [14].

3 - Testing the hole board

The exploration reaction is one in relation with both curiosity and the desire to escape from the animal. The material used is a plank against plated 40 x 40 cm and 1.8 cm thick. In this plate are drilled 16 holes 3cm in diameter, evenly

spaced. The plate is placed on the four legs of a stool returned, so that for mice, holes appear background sounds. Mice were placed one by one at the center of the board and one counts the number of times the mouse head plunges into a hole. The number of explored holes was observed after 1,2,3, 4 and 5 minutes. Averages are calculated for each minute and all 5 minutes.

For the duration of the trial, observers must maintain absolute silence and strictly remain motionless [15-20].

4 - Finding the hypnotic action

Animals that received the hypnotic lost their righting reflex. Laid back, they keep this position in contrast to the control group who turns and are measured:

Sleep latency: the time between the injection of the hypnotic and the abolition of the righting reflex (TE).

Sleep time: the time between the loss of righting reflex and its reappearance (TS).

5 - Search the catatonigene

The tests usually used to search for a catatonigene activity highlight catalepsy which is characterized in animals by a combination of loss of motor initiative, over the plasticity of the animal being considered cataleptic if they agree to pass forelegs with ipsilateral hind legs. It follows the evolution of catalepsy every 15 minutes. [15]

6 - Drug interaction study

The hypnotic activity of a substance can be confirmed by potentiations effect vis-à-vis a known reference.

For each test, three groups of 5 animals (mice or rats) are used:

Control group: receiving the solvent.
Lot Reference: receiving Urbanyl.
Group treated by drugs.

The doses of products **1**-**4** used are less than the LD_{50}, for mice; 200, 300 and 600 mg /kg in rats 600 mg / kg, 900 mg / kg (cataleptic hypnotic and tests), 20 mg / kg and 60 mg / kg for drug interaction.

RESULTS

a- ***Acute toxicity:***

Analysis of the results obtained shows that the LD_{50} value for compound **1** is 1.200 mg /kg in the case of upper and mouse 1200 mg / kg (ip) in the case of rats. For products **2** and **3**, the LD_{50} is 900 mg /kg (ip) in mice and higher than 1200 mg /kg (ip) in rats, then for compound **4**, the LD_{50} is greater than 1200 mg / kg (ip) for both mice and rats. Product **4** is less toxic than products **1**-**3 (**Table 2).

Evolution of weight after injection:

Monitoring the weight change of the animals used during the period of observation (14 days) shows a decrease in body weight 2-3 days after injection. Then when the apparent signs of toxicity begin to disappear, weight increases gradually.

Table 2: Acute toxicity (LD_{50} in mg/kg) of products 1-4

Animal	**1**	**2**	**3**	**4**
Mouse	1200	900	900	>1200
Rats	> 1200	>1200	>1200	>1200

b- Psychotropic activity

The results are expressed relative to the control batch and relative to the reference batch.

Tensile test:

Recovery time of the lot treated mice is extended by 30 min after administration compared to mice of batch control so that the mice treated with the product suffered three falls at 400 mg / kg dose.

Test fire:

In animals treated with the products tested, there was a loss of initiativity and curiosity. For a 400 mg /kg the animal did not try to mount the tube to escape (Table 3).

Test hole board:
The products tested reduced the cumulative number of explored holes (related curiosity) and the number of distances traveled between holes (related to motor activity).

Hypnotic effect:
The tested products have no hypnotic effect in rats.

Cataleptic power:
There was no catalepsy in rats treated with the three products tested.

Drug interactions:
Products **2**, **3** and **4** extend the hypnotic effect of Tranxene while product **1** gives an agitation which prevents animals to have a sound sleep (Table 4).

Table 3: Sedative effects of products 1- 4, tm1: average decay time, tm2 average recovery time

		witness	reference	1		2		3		4	
			80 mg/kg	300	600	300	600	300	600	300	600
traction	Number of falls	0	N=5 5	0	0	0	0	2	3	0	3
	Tm1 falls in second	0	N=5 (4±1)	0	0	0	0	N=2 (6±1)	N=3 (3±1)	0	45
Chimneye	Tm2 recoveries	N=5 (5±1)	0	N=5 (3±1)	N=5 (7±1)	N=5 (5±1)	N=5 (6±1)	N=3 (10±1)	N=2 (11±1)	N=2 (16±2)	N=5 (30±4)
	Positive response	5	0	2	0	0	0	0	0	0	0
Fishing Holes	Tm to raise the tube	N=5 (7±1)	N=5 T>2min	N=2 26±1 n=3 t>2min	N=5 t>2min	N=5 t>2min	N=5 t>2min	N=5 t>2min	N=5 t>2min	N=5 t>2min	N=5 t>2min
	Holes explored in 5min cours	N=5 (7±0)	N=5 (1±0)	N=5 (2±1)	N=5 (1±0)	N=5 (1±0)	N=5 (1±0)	N=5 (1±0)	N=5 (1±0)	N=5 (2±2)	N=5 (1±0)

Table 4: Drug Interaction of product 2 (TE: average asleep time; TS average sleep time)

		80 mg/kg of ranxene	80 mg/kg de Tranxene + 40 mg/kg of Urbanyl	80 mg/kg de Tranxene + 60 mg/kg of product **2**
		Witness	Reference	Product 2
Medication interaction	T.E	(20±2)	(18±2)	(15±2)
	T.S	(20±3)	There agitations during sleep.	(40±5)

DISCUSSION

All the results suggest that benzodiazepin-2-ones tested were not toxic and exert a sedative effect on the central nervous system. Compared to URBANYL, the products tested have less sedative effects. The benzyl group attached to the carbon (C3) in position 3 of the benzodiazepine ring (**4**) decreases the toxicity and sedative exerts a greater activity on the central nervous system. These results are similar to those observed by Zellou et al [21-22] and Keita et al [23] in the evaluation of the toxicity and psychoactive derivatives of 1,5-benzodiazepines synthesized in our laboratory.

CONCLUSION

We have studied the psychotropic activity of 4-acetonylidene-1,5 benzodiazepine -2 -one and its alkylated derivatives. The results indicate that the products tested were not toxic at therapeutic doses and exert a sedative effect on the central nervous system through a battery of behavioral tests used in psychopharmacology. When compared to the reference product, compound **4** has a greater sedative effect than the other products tested. We have also shown that the dialkylated products show a greater activity than monoalkylated products.

Acknowledgements
This study was supported by the Service of Cooperation and Cultural Action, SCAC of the France embassy in Nouakchott.

REFERENCES

[1] D.L-Loyd; H.P.Cleghorn, *Advances in Heterocyclic Chemistry* , Vol(17),**1974**, 27-43.
[2] G.A.Archer; L.Sternabach, *Chem,Rev*, **1968**, 771
[3] Y.okamoto; K.Takagi, *J.Heterocyclic, Chem*., **1987**, 24, 885
[4] Sidya.M, Synthèse, réactivité et étude pharmacologique de la 4- acétonylidène-1,5-benzodazépin-2-one. Thèse de Doctorat. Université Mohamed V, **2004**
[5] O.M. Sidya, B. Rachid, N. Joly, V. Lequart, P. Martin, M. Massoui , E.M. Essassi; molbank, **2006**, M499.
[6] O.M. Sidya ; B. Djerrari ; M. El Abbassi, ; J. Fifani ; E.M. Essassi, B. El-Bali ; M. Bolte, *Acta Cryst*, **2000**. C(56), 165-166
[7] M. Broustail.. La Souris de Laboratoireet son Elevage (3éme éd). Vigot Frères: Paris, **1967**.
[8] M.J. Laroche ; F. Rousselet. Les Animaux du Laboratoire : Ethique et Bonnes Pratiques. Masson, Paris, **1990**.
[9] D. Ernest ; DVM Olfert ; M. Brenda ; DVM. Cross ; A.M. William. , Manuel sur le Soin et l'Utilisation des Animaux d'Expérimentation, Conseil Canadien de Protection des Animaux, **1993**.
[10] J.T. Litchfield ; Wilcoxon, *J. Pharmacol* ,**1970** , 3, 407-414..
[11] M.J. Laroche; P. Fabiani, ; F. Rousselet, Expertise Toxicologique des médicaments, 11, Masson, Paris, 1986.
[12] J. Wepierre, Abrégé de pharmacologie générale et moléculaire Masson, Paris, **1981**.
[13] Couvoisiers, R.Duewt, L. Joulan. *Psychotropic Drugs : eds Garathinis. Ghettiv, Amesterdam* **1957** , 373 – 391.
[14] R. Hazard ,J. Cheymoz. Mannuel de pharmacologie Masson et Cie éditeurs, Paris, **1963**.
[15] J.T. Boissier ; P. Simon, *Thérapie* **1962** , 17 , 1225-1232.
[16] J.R. Boissier ; P. Simon, J.M. Lwoff, *Thérapie* **1964**,19, 571-589.
[17] J.R. Boissier ; P. Simon, *Thérapie* **1966**, 21, 799-818.
[18] J.R. Boissier; P. Simon, *Thérapie* **1967**, 22, 467-468.
[19] J. Driessech ; J.B. le polles , *J.Pharmacol*, **1978**, 9, 277-284.
[20] J. R. Boissier ; P. Simon. *Thérapie*, **1963**, 18, 1257 – 1277.
[21] A. Zellou ; Y. Cherrah ; M. Hassar ; EM Essassi., *Ann Pharm.Fr* **1998** ; 56 (4) 169-174.
[22] A. Zellou ; Y. Cherrah ; E.M. Essassi ; M. Hassar, *Ann Pharm.* Fr **1998** ; 56 (4) 175-180.
[23] A. Zellou ; A. Keita , Y. Cherrah ; E.M. Essassi ; M.Hassar, *J. Therapie* **1999** , 19 , 645-649.