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NEW PHTHALIMIDE DERIVATIVES WITH POTENT ANALGESIC ACTIVITY: II

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Abstract: Seven new phthalimide derivatives (**9a~g**) with 1,2,4-oxadiazol-5-yl methyl group attached to nitrogen have been synthesized from *N*-phthaloylglycine **6** and arylamidoximes **7a~g**. All of these showed potent analgesic effect with acetic acid induced "writhing" test in mice. One of the better compounds (ID_{50} = 2.2 mg/Kg ip) has been found to be **9a** which also demonstrates analgesic activity against inflammatory pain.

Nonsteroidal analgesic and antiinflammatory drugs have been the subjects of intensive research for a long time, and the search for better, more effective, and less or nontoxic drugs still continues. Many of these drugs known today have both analgesic and antiinflammatory properties,[1] for example, salicylic acid derivatives, *para*-aminophenol derivatives, indole and indene acetic acids, and arylpropionic acids (Ibuprofen®). The nonsteroidal antiinflammatory drugs (NSAIDs) inhibit the cyclooxygenase enzyme (COX or PGHS), which is responsible for the metabolization of arachidonic acid to prostaglandins, which cause inflammation and pain. Therefore, the mechanism of action of compounds with analgesic and antiinflammatory properties is thought to be due to the inhibition of prostaglandin synthesis.[2] Although this proposed mechanism is commonly accepted, recent data suggests that relief of pain by NSAIDs may occur via a mechanism other than the inhibition of prostaglandin synthesis, including antinociceptive effects at peripheral and central neurons.[3,4]

For several years the authors have been looking for compounds with potential analgesic and antiinflammatory properties. In fact, one of the propionic acids, 3-[3-(phenyl)-1,2,4-oxadiazol-5-yl]propionic acid (POPA), prepared by us showed both of these properties.[5] We have also been interested in phthalimide derivatives, and a search of literature revealed that thalidomide (although withdrawn from the market) was remarkably effective for the treatment of erythema nodusum leprosum (ENL), an acute inflammatory-manifestation of lepromatous leprosy.[6,7] More recently, thalidomide was found to exert immunomodulatory and antiinflammatory effects.[8] It was decided, therefore, to synthesize compounds having phthalimide and 1,2,4-oxadiazol moieties in one molecule and evaluate their biological activities. In a recent communication,[9] the synthesis and semi-empirical molecular orbital calculations (AM1) of three

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N-[3-(phenyl)-1,2,4-oxadiazol-5-yl alkyl]phthalimides **1~3** (Fig. 1), were reported and predicted that these might show antihyperlipidemic activity. Besides these three phthalimido-oxadiazoles, the literature does not record any more compound of this series.

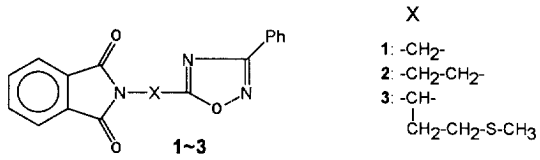


Fig. 1

Based upon the results described above, it was decided to prepare more compounds of this series and evaluate their biological activities. In this paper, we describe the synthesis and analgesic properties of seven new phthalimido-oxadiazoles **9a~g** (Fig. 2). Interestingly, all of them exhibit good analgesic properties.

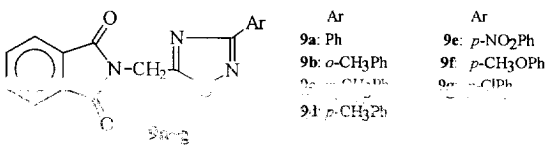
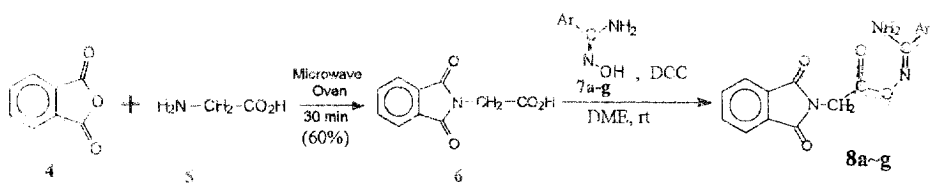


Fig. 2

Reaction of glycine **5** with phthalic anhydride **4** in a domestic microwave oven provided *N*-phthaloylglycine **6** with a yield of 60% (Scheme 1). Although the preparation of **6** has been reported,[10] its synthesis from **4** and **5** using a microwave oven has not been described in the literature. However, a new method of synthesis of N-alkylphthalimide via alkylation of phthalimide under microwave irradiation has been reported.[11]



Scheme 1

The spectral data agreed with the structure of **6**. Reaction of **6** with arylamidoximes **7a~g** in the presence of dicyclohexylcarbodiimide (DCC) at room temperature affords the intermediate **8a~g** (Scheme 1). Although these may be isolated, no efforts have been made to purify them because of the possibility that a certain percentage of the intermediate may cyclize to give **9**. Their structures are assumed to be **8a~g**, because it has been established that arylamidoximes and a carboxylic acid in presence of DCC react to give an *O*-acyl intermediate.[12] On the TLC (chloroform/ethyl acetate, 85/15), these have slightly higher R_f values (0.2) than aryl amidoximes ($R_f = 0.1$).

Heating of **8a~g** individually for an extended period of time furnished **9a~g**. The title compounds are remarkably stable. Their structures have been determined with the help of elemental analyses and spectral data.

Phthalimido-1,2,4-oxadiazoles are a new class of compounds and therefore no pharmacological data is available. The literature reports on the fungicidal activity of 2-(phthalimido-methylamino)-5-aryl-1,3,4-oxadiazoles.[13] Therefore, compounds **1~3** and **9a~g** have potential interest for a series of biological activity tests. As a first step, **9a~g** were tested for analgesic properties and found to possess such activity. These results and their discussion are given below.

Eventhough no LD_{50} (lethal dose 50%) values were determined, none of the 1,2,4-oxadiazoles caused death in mice when administered in doses up to 200 mg/kg intraperitoneally (ip), which indicates that the analgesic activity is produced in nonlethal doses. However, this does not necessarily mean that these substances are nontoxic. The inhibition of the abdominal constriction response (writhing) induced by acetic acid is a commonly employed test for detecting analgesic activity of organic compounds.[14] The antinociceptive property of these substances needs to be confirmed in other experimental models as nonanalgesic agents have been reported to inhibit the abdominal constriction response.[15]

Table 1. Inhibition of acetic acid induced writhing in mice.

Compound	Dose (mg/kg)	No. of tests	Inhibition of 'writhing' (%)	ID_{50} (with 95% confidence limit)	Compound	Dose (mg/kg)	No. of tests	Inhibition of 'writhing' (%)	ID_{50} (with 95% confidence limit)
Aspirin	200	3	74.0			12.5	4	72.5*	
	100	3	61.7*	80.8 (50.8-103.0)	9d	6.25	4	62.4*	7.2 (4.8-10.8)
	50	3	38.20*			3.12	3	16.1$^{N.S}$	
	6.25	4	73.8*			6.25	3	62.9*	
9a	3.12	3	67.1*	2.2 (1.4-3.5)	9e	3.12	3	47.4*	4.1 (2.4-7.0)
	1.56	3	38.9*			1.56	3	29.8$^{N.S}$	
	6.25	4	80.5*			25.0	3	62.6*	
9b	3.12	3	76.5*	3.1 (2.1-4.6)	9f	12.5	3	53.3*	14.9 (7.5-29.8)
	1.56	3	12.1$^{N.S}$			6.25	3	41.9*	
						3.12	3	20.7$^{N.S}$	
	6.25	4	79.2*			25.0	3	59.6*	
9c	3.12	3	67.1*	2.9 (1.9-4.4)	9g	12.5	3	35.1$^{N.S}$	20.3 (11.3-36.5)
	1.56	3	25.5*			6.25	3	16.3$^{N.S}$	

N.S = not significant * =significant

Table 1 shows a summary of the results from which it can be observed that oxadiazoles **9a~c** have approximately similar activity when their respective ID_{50} (inhibitory dose 50%) values are compared, while **9d** is less than half as active. It is surprizing to note that substitution of the phenyl ring at *para* position decreases the activity.

The substances tested are analgesics and these are drugs that relieve pain without loss of consiousness. Lin et al.[16] grouped them into three categories: (a) peripherally acting, non-narcotic; (b) centrally acting, non-narcotic; and (c) centrally acting, narcotic.

It is less likely that the present phthalimides have much effect on the central nervous system, further experiments using different models of analgesia are required to place the present compounds in their most appropriate category. It was found that these compounds have much better activity than aspirin. On the other hand, preliminary tests carried out with **9a**, employing the formalin test in mice,[17,18] which showed an effect of pain in two phases: initial or no inflammatory and late or inflammatory. With this test, we tried to identify if this substance inhibits the pain at inflammatory or noninflammatory phase. Hunskaar and Hole[19] showed that morphine and aspirin, for example, inhibited both phases, and in contrast, NSAIDs like indomethacin and naproxen and the steroids dexamethasone and hydrocortisone are only late phase inhibitors. The results of Table 2 show that there is an action of **9a** at late phase of the test, indicative of analgesic activity on inflammatory pain. In the near future, substances **9a~g** will be tested for antiinflammatory activity by the classic method of rat paw edema induced by carrageenan.[20] We are now trying to understand the mechanism of action of these oxadiazolo-phthalimides. Potentially, these substances **9a~g** act as NSAIDs, and, so we intended to extend our studies using COX-1 and COX-2 enzymes, and to discover if these substances inhibit one or both isoforms.

Table 2. Hyperalgesia in mice induced by formalin: dissociation between inflammatory and non-inflammatory pain.

		Time of licking(s)*	
Substance	**Dose (mg/kg)**	**Initial phase (up to 5 min)**	**Late phase (up to 30 min)**
Control	10 (mL/kg)	84,4 ± 9,6	143,1 ± 15,1
9a	25	84,8 ± 8,3	75,3 ± 8,7
Aspirin	400	48,8 ± 5,9	0
Morphine	10	41,3 ± 3,9	0

*These are average values of three tests with groups of eight mice.

Pharmacology. The compounds **9a~g** in different doses and aspirin at 100 mg/kg dose were suspended in 0.2% Tween 80. Each solution was administered ip 1 hour before the ip injection of 0.75% acetic acid

solution (0.1 mL/10 g) to a group of 5 mice (23~28 g). The number of abdominal constrictions produced in each group for succeeding 15 min was counted and compared with the response in the control group as described by Heapy et al.[21] Each dose of the compound was tested three to six times and the mean values were calculated. The inhibitory dose 50% (ID_{50}) was obtained from dose~response curves and statistical analysis of the results was considered significant when $p < 0.05$ (by using Student's t-test). The substance **9a** (25 mg/kg) as a suspension in Tween 80, was administered intraperitoneally (ip). After 30 min, 20 μL of 1% formalin was injected subcutaneously into the dorsal hind paw of mice. The mice was then put back into the chamber where the observation period started. The time was registered in seconds, where the mice spent licking the injected paw. Two distinct periods of intensive licking activity were identified and scored separately.[19] The control was a 0.9% saline solution (10 mL/kg), ip and standards was aspirin (400 mg/kg) and morphine (10 mg/kg), both ip Each dose of compounds was tested three time and the average values as well as the standard error was calculated.

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