Synthesis and analgesic activity of N-arylhydrazone derivatives of mefenamic acid

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Abstract: **PURPOSE**: A series of N-Arylhydrazone derivatives of mefenamic acid (a known non-steroidal anti-inflammatory drug) were synthesized in order to obtain new compounds with potential analgesic and anti-inflammatory activity. **METHODS:** The structures of all synthesized compounds were confirmed by means of infrared, proton magnetic resonance and mass spectroscopy. All compounds were evaluated for their analgesic and anti-inflammatory activities by abdominal constriction test (writhing test) and carrageenan-induced rat paw edema test respectively. **RESULTS**: Most of the synthesized compounds induced significant reduction in the writhing response when compared to control. Among them, compounds **11, 12, 15, 16, 19, 20,** and **21** were significantly more potent than mefenamic acid in the writhing test. The anti-inflammatory activity of these 7 compounds were evaluated and compounds **11, 12, 16, 19** and **20** showed significant anti-inflammatory activity in comparison to control but their effect was weaker than mefenamic acid. **CONCLUSIONS:** The antinociceptive relative activity of some of these newly synthesized compounds is greater than mefenamic acid but they are not potent anti-inflammatory agents.

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INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDS) are widely used in the treatment of pain and inflammation. These compounds non selectively inhibit the two isoforms of the cyclooxygenase (COX-1 and COX-2) and thus prevent the metabolism of cellular arachidonic acid (AA) and the upregulation of prostaglandin formation, which otherwise lead to an increase of vascular permeability, edema, hyperalgesia, pyrexia and inflammation. In addition to COX, the 5-lipoxygenase (5-LO) enzyme is another key enzyme which is involved in the AA cascade. Leukotrienes, produced through the 5-LO enzyme pathway, may also contribute to both inflammation and NSAIDs induced side effects. For these reasons, compounds that are dual inhibitors of both COX and 5-LO are being studied as potential analgesic and anti-inflammatory agents with an improved safety profile in comparison to NSAIDS (1-2). Currently, various chemical families of dual COX/5-LO inhibitors can be found in the scientific literatures (3). In the 1980's, hydrazone-type containing compounds such as BW 755c (**1**) and CBS 1108 (**2**) (Fig 1) were described as dual COX/5-LO inhibitors which present analgesic and anti-inflammatory activities.

In fact, some evidences suggest that the hydrazone moiety present in derivative **3** (Fig 1) possess a pharmacophoric character for the inhibition of COX. According to these results, analgesic profile of new series of heterocyclic N-acylarylhydrazones **4-6** (Fig 2) has been previously described (4-5).

In addition to these compounds, there are some reports about importance of fenamate structures in dual inhibition of COX/5-LO by substitution of their carboxylic acid moiety with some acidic heterocycles, namely 1,3,4-oxadiazole-2-thione (**7**) and 1,3,4-thiadiazole-2-thione (**8**) (Fig 3) eg in mefenamic acid (1,6).

Thus, we decided to replace the carboxylic acid moiety of mefenamic acid, a known NSAID drug, with an N-arylhyrazone group in the hope of obtaining additional inhibitors of cellular AA metabolism.
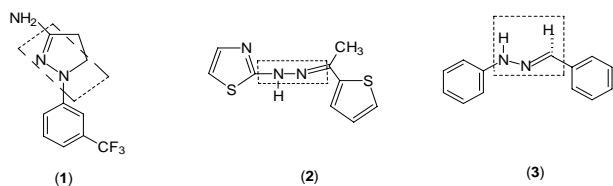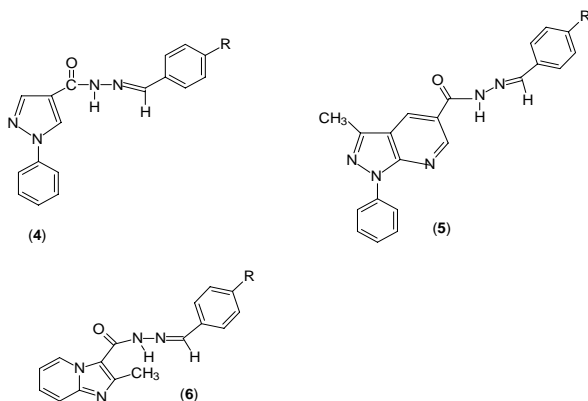
Figure 1: Structures of Compounds **1-3**.

Figure 2: Structures of compounds **4-6**.

The target compounds were synthesized by acid-catalyzed condensation of **9** with the corresponding aromatic aldehydes (Scheme 1) (4).
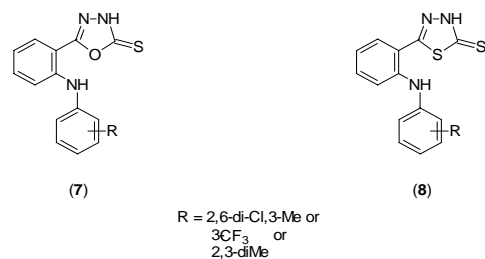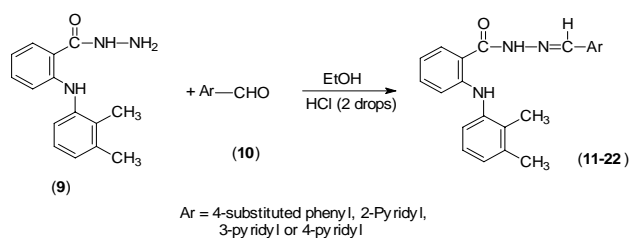

R = 2,6-di-Cl,3-Me or
3CF₃ or
2,3-diMe

Figure 3: Structures of compounds **7** and **8**.

Ar = 4-substituted phenyl, 2-Pyridyl,
3-pyridyl or 4-pyridyl

Scheme 1: Synthesis of target compounds

MATERIAL AND METHODS

Chemistry

Chemicals were purchased from Merck Chemical Company (Darmstadt, Germany). Melting points were taken on a Kofler hot stage apparatus (Reichert, Vienna, Austria) and are uncorrected. ^{1}H-NMR spectra were obtained using a Brucker FT-80 spectrometer (Brucker, Rheinstetten, Germany). Tetramethylsilane was used as an internal standard. Mass spectra were obtained using a Finnigan Mat TSQ-70 spectrometer at 70 ev (Finnigan Mat, Bremen, Germany). The IR spectra were obtained using a Nicolet FT-IR Magna 550 spectrographs (KBr disks) (Nicolet, Madision, WI, USA). The purity of compounds was confirmed by TLC using different mobile phases. The results of the elemental analyses (C, H, N) were within±0.4% of theoretical values for C, H and N.

Synthesis of N-arylhdyrazone derivatives of mefenamic acid (11-22).

The hydrazide derivative of mefenamic acid (**9**) (Scheme 1) was prepared according to the previously described methods (7-8).

A mixture of 1.9 mmol of hydrazide **9** and 1.9 mmol of the corresponding aldehyde derivative **10** in 20 ml of absolute ethanol was stirred at room temperature for 0.5 to 1h, in the presence of two drops of hydrochloric acid as a catalyst. The end of the reaction was observed by TLC, and the hydrazones **11-22** were isolated by concentration of the reaction mixture under reduced pressure, followed by neutralization with a 10% aqueous solution of sodium bicarbonate. The resulting precipitate was filtered, washed with 10 ml water and crystallized from a suitable solvent. Melting points, crystallization solvents and yields are reported in Table 1.

The spectral data of new synthesized compounds are as follows: **Compound 11**:- IR (KBr): ν cm^{-1} 3319 (NH), 3196 (NH), 1634 (C=0). - ^{1}H-NMR (CDCl₃): δ (ppm) 9.12 (bs, 1H, NH), 9.07 (bs, 1H, NH), 8.11 (s, 1H, CH), 7.60 (t, 3H, aromatic), 7.14-6.46 (m, 8H, aromatic), 2.37 (s, 3H, CH₃), 2.31 (s, 3H, CH₃), 2.19 (s, 3H, CH₃).- MS: m/z (%) 357 (M^{+}, 68), 224 (100), 208 (30), 180 (17). **Compound 12**:- IR (KBr): ν cm^{-1} 3345 (NH), 3237 (NH), 1634 (C=0). -^{1}H-NMR (CDCl₃): δ (ppm) 9.18 (bs, 1H, NH), 9.08 (bs, 1H, NH), 8.16(s, 1H, CH), 7.82-7.45 (m, 3H, aromatic), 7.19-6.58 (m, 8H,

aromatic), 2.32 (s, 3H, CH$_3$), 2.19 (s, 3H, CH$_3$).- MS: m/z (%) 361 (M$^+$, 67), 224 (100), 207 (21), 179 (19). **Compound 13**: -IR (KBr) ν cm^{-1} 3298 (NH), 3211 (NH), 1629 (C=0). -^{1}H-NMR (CDCl$_3$): δ (ppm) 9.13 (bs, 1H, NH), 9.04 (bs, 1H, NH), 8.10 (s, 1H, CH),

7.77-7.50 (m, 3H, aromatic), 7.14-6.73 (m, 8H, aromatic), 3.84 (s, 1H, OCH$_3$), 2.32 (s, 1H, CH$_3$), 2.20 (s, 1H, CH$_3$).-MS: m/z (%), 373 (M$^+$, 48), 222 (100), 207 (20), 180 (11).

Table 1: Physical data of synthesized compounds
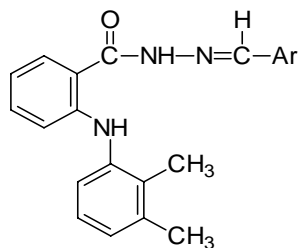
Compound No	Ar	MP°C	yield %	Recrystallization Solvent	Molecular Formula
11	4-tolyl	197-199	14	EtOAc/Petr-Ether	C$_{23}$H$_{23}$N$_3$O
12	4-fluorophenyl	195-197	35	EtOH	C$_{22}$H$_{20}$FN$_3$O
13	4-methoxyphenyl	216-217	68	EtOAc	C$_{23}$H$_{23}$N$_3$O$_2$
14	phenyl	192-193	24	EtOAc/Petr-Ether	C$_{22}$H$_{21}$N$_3$O
15	4-hydroxyphenyl	190-194	27	EtOAc	C$_{22}$H$_{21}$N$_3$O$_2$
16	4-nitrophenyl	221-222	62	EtOH	C$_{22}$H$_{20}$N$_4$O$_4$
17	4-chlorophenyl	204-206	43	EtOH	C$_{22}$H$_{20}$ClN$_3$O
18	4-N,N-dimethylamino-phenyl	247-248	44	EtOH	C$_{24}$H$_{26}$N$_4$O
19	4-pyridyl	203-204	76	EtOAc	C$_{21}$H$_{19}$N$_4$O
20	3-pyridyl	182-185	39	MeOH	C$_{21}$H$_{19}$N$_4$O
21	2-pyridyl	160-161	41	EtOAc	C$_{21}$H$_{19}$N$_4$O
22	4-Bromophenyl	212-215	90	MeOH	C$_{22}$H$_{20}$BrN$_3$O

Compound 14:- IR (KBr): ν cm^{-1} 3324 (NH), 3206 (NH), 1629 (C=0). -^{1}H-NMR (CDCl$_3$): δ (ppm) 9.32 (bs, 1H, NH), 9.15 (bs, 1H, NH), 8.17 (s, 1H, CH), 7.76-6.72 (m, 12H, aromatic), 2.32 (s, 3H, CH$_3$), 2.19 (s, 3H, CH$_3$).- MS: m/z (%) 343 (M$^+$, 55), 224 (100), 209 (17). **Compound 15**:- IR (KBr): ν cm^{-1} 3355 (OH), 3288 (NH), 3210 (NH), 1620 (C=0). -^{1}H-NMR (DMSO-d$_6$): δ (ppm) 11.05 (bs, 1H, OH), 9.5 (bs, 1H, NH), 9.32 (bs, 1H, NH), 8.33 (s, 1H, CH), 7.58-7.42 (m, 3H, aromatic), 7.21-6.68 (m, 3H, aromatic), 2.31 (s, 3H, CH$_3$), 2.15 (s, 3H, CH$_3$).- MS: m/z (%) 359 (M$^+$, 55), 224 (100), 208 (29), 180 (19). **Compound 16**:- IR (KBr) ν cm^{-1} 3355 (NH), 3280 (NH), 1639 (C=0), 1347 (NO$_2$), 1521 (NO$_2$). -^{1}H-NMR (CDCl$_3$): δ (ppm) 9.46 (bs, 1H, NH), 9.16 (bs, 1H, NH), 8.30 (s, 1H, CH), 8.24 (d, J=8.8 Hz, 2H, aromatic), 7.89 (d, J=8.8 Hz, 2H, aromatic), 7.55 (dd, J=7.9, 1.5 Hz, 1H, aromatic), 7.18-6.61 (m, 6H,

aromatic), 2.32 (s, 3H, CH$_3$), 2.19 (s, 3H, CH$_3$).- MS: m/z (%) 388 (M$^+$, 48), 222 (100), 208 (32), 180 (16). **Compound 17**:- IR (KBr): ν cm^{-1} 3329 (NH), 3196 (NH), 1629 (C=0). -^{1}H-NMR (CDCl$_3$): δ (ppm) 9.32 (bs, 1H, NH), 9.03 (bs, 1H, NH), 8.15 (s, 1H, CH), 7.73-6.69 (m, 11H, aromatic), 2.31 (s, 3H, CH$_3$), 2.19 (s, 3H, CH$_3$).- MS: m/z (%) 377 (M$^+$, 19), 222 (100), 207 (25), 180 (14).

Compound 18: -IR (KBr): ν cm^{-1} 3240 (NH), 3211 (NH), 1634 (C=0).-^{1}H-NMR (CDCl$_3$): δ (ppm) 9.10 (bs, 1H, NH), 8.95 (bs, 1H, NH), 8.03 (s, 1H, CH), 7.70-7.45 (m, 3H, aromatic) 7.15-6.64 (m, 8H, aromatic), 3.02 (s, 6H, N(CH$_3$)$_2$), 2.31 (s, 3H, CH$_3$), 2.20 (s, 3H, CH$_3$). –MS: m/z (%) 386 (M$^+$, 81), 223 (74), 163 (100), 40 (38). **Compound 19**: -IR (KBr): ν cm^{-1} 3355 (NH), 3217 (NH), 1639 (C=0). -^{1}H-NMR (CDCl$_3$): δ (ppm) 9.52 (bs, 1H, NH), 9.04 (bs, 1H, NH), 8.66 (d, 2H, J=6Hz, aromatic), 8.21

(s,1H, CH), 7.64-7.53 (m, 3H, aromatic) 7.15-6.70 (m, 6H, aromatic).- MS: m/z (%) 344 (M$^+$, 28), 224 (100), 209 (27), 180 (22). **Compound 20**: -IR (KBr): ν cm^{-1} 3331(NH), 3192 (NH), 1625 (C=0). -^{1}H-NMR (CDCl$_3$): δ (ppm) 9.78 (bs, 1H, NH), 9.05 (bs, 1H, NH), 8.78 (s, 1H, aromatic), 8.60 (d, J=6.2 Hz, 1H, aromatic), 8.27 (s, 1H, CH) 8.13 (d, J=6.2 Hz, 1H, aromatic), 7.58 (d, J=7.9 Hz, 1H, aromatic), 7.37-6.67 (m, 7H, aromatic), 2.31 (s, 3H, CH$_3$), 2.17 (s, 3H, CH$_3$).- MS: m/z (%) 344 (M$^+$, 57), 224 (100), 209 (29), 180 (26), 120 (16). **Compound 21**: -IR (KBr): ν cm^{-1} 3283 (NH), 3190 (NH), 1632 (C=0). -^{1}H-NMR (CDCl$_3$): δ (ppm) 9.41 (bs, 1H, NH), 9.20 (bs, 1H, NH), 8.71-8.47 (m, 3H, aromatic), 8.22 (s, 1H, CH), 7.95-6.55 (m, 8H, aromatic), 2.28 (s, 3H, CH$_3$), 2.14 (s, 3H, CH$_3$). –MS: m/z (%) 344 (M$^+$, 95), 224 (100), 208 (22), 180 (19), 120 (68), 92 (40).

Compound 22: -IR (KBr): ν cm^{-1} 3331 (NH), 3169 (NH), 1622 (C=0).-^{1}H-NMR (CDCl$_3$): δ (ppm) 11.55 (bs, 1H, NH), 9.21 (bs, 1H, NH), 8.37 (s, 1H, CH), 7.72-7.43 (m, 5H, aromatic) 7.28-6.56 (m, 6H, aromatic), 2.31 (s, 3H, CH$_3$), 2.19 (s, 3H, CH$_3$). –MS: m/z (%) 422 (M$^+$, 21), 223 (100), 210 (28), 180 (25).

Pharmacology

Male NMRI mice weighing 20-25 g and male Wistar rats weighing 200-250 g (from animal house of Faculty of Pharmacy, TUMS) were used for the abdominal constriction test (writhing test) and the carrageenan-induced paw edema respectively. The animals were housed in colony cages and conditions of constant temperature (22 ± 2°C) and a 12 h light/dark schedule and allowed free access to standard diet and tap water except during the experiment. The animals were allowed to habituate to the laboratory environment for 2h before the experiments were initiated. All ethical manners for use of laboratory animals were considered carefully and the protocol of study was approved by TUMS ethical committee. The compounds were administered intraperitoneally (IP) (31 μmol/kg; 0.2 ml/20g) as a suspension in saline and tween 80 (4% w/v). Mefenamic acid (Hakim Pharmaceutical Co) (31 μmol/kg, IP) (9) was used as standard drug under the same conditions. The control group received vehicle (0.2 ml/20g, IP) alone.

Analgesic Activity

The analgesic activity was determined in vivo by the abdominal constriction test induced by acetic acid (0.6%; 0.1 ml/10g) in mice (10). An acetic acid solution was administered IP 30 minutes after administration of compounds. Antinociception was recorded by counting the number of writhings immediately after injection of acetic acid during 30 minutes. The analgesic activity was expressed as the percentage of inhibition of constrictions when compared with the vehicle control group Table 2.

Anti-inflammatory activity

The anti-inflammatory activity was determined in vivo using the carrageenan-induced rat paw edema test (5, 11). A solution of 0.1 ml of 1% carrageenan (Sigma-Aldrich, Dorset, UK) in saline was injected sub plantarly in the right hind paw of the rats 1h after IP administration of compounds. The paw thickness was measured from the ventral to the dorsal surfaces using a dial caliper immediately prior to carrageenan injection and then at each hour, up to 4 h after the sub planar injection. The edema was calculated as the thickness variation between the carrageenan and saline treated paw. Anti-inflammatory activity was expressed as the percent of inhibition of the edema when compared with the control group.

Statistics

The results are expressed as the mean ± SEM of *n* animals per group. The data were statistically analyzed by one way analysis of Variance (ANOVA) followed by Tukey multicomparison test. differences with P<0.05 between experimental groups were considered statistically significant.

RESULTS

All new N-arlyhydrazone derivatives of mefenamic acid (**11-22**) were initially evaluated for analgesic activity using the acetic acid induced mice abdominal constrictions test and the results are shown in Table 2.

Except compounds **14**, **17**, **18** and **22**, all of them induced significant reduction in the writhing response in comparison to control and among them 7 compounds significantly showed higher inhibitory effect on the writhing response in comparison to mefenamic acid as follow: (**11**, 71.1%, P<0.001) (**12**, 58.5%, P<0.001), (**15**, 46.7%, P<0.01), (**16**, 42.4%; P<0.01), (**19**, 67.9%; P<0.001), (**20**, 93.7%, P<0.001), (**21**, 50.9%, P<0.01), mefenamic acid (25.6%, P<0.01).

Table 2: Effects of Compounds 11-22 and mefenamic acid in the abdominal constrictions induced by acetic acid in mice

Compound	Dose (μmol/kg)[1]	Constriction No. (mean ± SEM)	Inhibition (%)[2]	Relative activity[3]	P value
Control	-	70.33 ± 3.00	-	-	-
mefenamic acid	31	52.33 ± 2.59	25.59	1	P<0.01 vs. control
11	31	20.33 ± 4.62	71.09	2.78	P<0.001 vs. control P<0.001 vs. ma[4]
12	31	29.20 ± 3.73	58.45	2.28	P<0.001 vs. control P<0.001 vs. ma
13	31	47.50 ± 4.37	32.45	1.27	P<0.01 vs. control P>0.05 vs. ma
14	31	65.00 ± 4.54	7.57	0.30	P>0.05 vs. control P<0.05 vs. ma
15	31	37.50±2.32	46.67	1.82	P<0.001 vs. control P<0.01 vs. ma
16	31	40.50±2.59	42.41	1.66	P<0.001 vs. control P<0.01 vs. ma
17	31	65.17±5.11	7.34	0.29	P>0.05 vs. control P<0.05 vs. ma
18	31	66.00±3.28	6.16	0.24	P>0.05 vs. control P<0.05 vs. ma
19	31	22.60±4.17	67.86	2.24	P<0.001 vs. control P<0.001 vs. ma
20	31	4.4±2.01	93.70	3.66	P<0.001 vs. control P<0.001 vs. ma
21	31	34.50±5.57	50.94	1.99	P<0.001 vs. control P<0.01 vs. ma
22	31	71.33±5.57	-1.42	-5.5	P>0.05 vs. control P<0.05 vs. ma

[1] number of animals in each group n= 6;[2] % inhibition obtained by comparison with vehicle control group; [3] Analgesic activity relative to mefenamic acid 4 ma (mefenamic acid).

Since these 7 compounds showed more analgesic activity in comparison to mefenamic acid, we decided to evaluate their anti-inflammatory profile by using the carrageenan induced rat paw edema test and the results are summarized in Table 3.

Compounds **11**, **12**, **16** and **19** as well as mefenamic acid as the reference drug induced significant anti-inflammatory activity after 3 and 4 h in comparison to control but none of the tested compounds was more active than mefenamic acid. Compound **20** showed significant anti-inflammatory activity after 1, 2 and 3h in comparison to control and after 1 h in comparison to mefenamic acid.

DISCUSSION

The pharmacological results of the present study show a good analgesic profile in comparison to control and mefenamic acid. Similar to the previous studies the most active derivatives **19, 20** and **21** possess the pyridine ring at the aryl moiety of the arylhydrazone frame work (4). In addition the compounds possessing the 4-tolyl or 4-flurophenyl moiety **11, 12** respectively are among the most active compounds in our study. Previous studies indicated that 4-Bromophenyl and 4-N, N-dimethyl-aminophenyl have a major contribution to the analgesic activity (4-5, 12-13) but our

pharmacological evaluations showed opposite results. Compounds **18**, **22** are among the weakest synthesized structures. As we described some N-arylhydrazone derivatives **4-6** (Fig 2) have presented an important analgesic profile which found to be more influenced by the nature of phenyl ring substituent of the hydrazone sub-unit than the pattern of the heterocyclic ring of the N-acyl moiety (4). Therefore, it is possible that replacement of these kinds of acyl groups with a fenamate structure has changed the mechanism of enzyme-receptor interaction and the importance of 4-substituents of phenyl rings at the aryl moiety of the aryl hydrazone frame work. Since in vivo activity depends on highly complex physiological interactions, therefore at this moment we are unable to rationalize all of these pharmacological results.

Table 3: Effects of compounds (11, 12, 15, 16, 19, 20, 21) and mefenamic acid in the inhibition of carrageenan-induced rat paw edema

Compound	Time (h)[1]	Dose (μml/kg)[2]	Thickness variation (mm)[3]	Inhibition (%)[4]	p value[5]
Control	1	-	1.062±0.123	-	-
	2	-	2.072±0.157	-	-
	3	-	2.748±0.161	-	-
	4	-	2.413±0.300	-	-
mefenamic acid	1	31	1.00±0.118	5.8	P>0.05
	2	31	1.937±0.258	6.5	P>0.05
	3	31	1.412±0.300	48.6	P<0.01
	4	31	0.915±0.202	62	P<0.01
11	1	31	1.497±0.289	-40.9	P>0.05
	2	31	1.817±0.271	12.3	P>0.05
	3	31	1.697±0.297	38.2	P<0.05
	4	31	1.370±0.351	43.2	P<0.05
12	1	31	1.327±0.098	-24.9	P>0.05
	2	31	2.173±0.183	-4.9	P>0.05
	3	31	1.865±0.069	32.1	P<0.001
	4	31	1.265±0.250	47.57	P<0.05
15	1	31	0.910±0.187	14.3	P>0.05
	2	31	1.770±0.137	14.57	P>0.05
	3	31	2.265±0.172	17.57	P>0.05
	4	31	2.212±0.329	8.33	P>0.05
16	1	31	1.525±0.229	-43.6	P>0.05
	2	31	2.635±0.244	-27.2	P>0.05
	3	31	1.665±0.178	39.4	P<0.01
	4	31	1.450±0.201	39.9	P<0.05
19	1	31	1.120±0.218	-5.5	P>0.05
	2	31	1.770±0.069	14.3	P>0.05
	3	31	1.723±0.157	37.3	P<0.01
	4	31	1.480±0.129	38.67	P<0.05
20	1	31	0.512±0.105	51.8	P<0.01
	2	31	1.330±0.237	35.8	P<0.05
	3	31	1.960±0.213	28.67	P<0.05
	4	31	2.250±0.336	6.75	P>0.05
21	1	31	1.375±0.131	-29.47	P>0.05
	2	31	2.433±0.183	-17.42	P>0.05
	3	31	2.495±0.131	9.2	P>0.05
	4	31	2.067±0.148	14.3	P>0.05

[1] *Time after carrageenan injection (0.1 mg/paw);* [2] *number of animals in each group n=6;* [3] *Thickness variation is the difference between the thickness of carrageenan-treated paw and saline-treated paw;* [4] *percentage of inhibition obtained by comparison with the vehicle;* [5] *p value of all compounds except the first hour of compound 20, the third and fourth hours of compound **21** in comparison to mefenamic acid was P>0.05; results are expressed as mean ± SEM.*

The anti-inflammatory evaluation of 7 most potent compounds showed that replacement of carboxylic acid group of mefenamic acid with N-arylhydrazone moiety can not produce any advantage in the anti-inflammatory properties of this drug. Most of the synthesized compounds had a similar bioavailability profile to mefenamic acid because this reference drug and compounds **11**, **12**, **16** and **19** were active after 3 and 4 h in comparison to control. Compound **20** showed significant anti-inflammatory effect only after 1h in comparison to mefenamic acid and in the first 3 h in comparison to control. The effect of compound **20** decreased stepwise from the first hour to the forth hour. Therefore in spite of a relative high potency after 1h it does not have a good kinetic profile.

We can deduce from the results that replacement of the acidic moiety of mefenamic acid with N-arylhydrazone moiety can create potent analgesic compounds. Further studies are needed to explore the differences in the efficacy and safety of synthesized compounds.

ACKNOWLEDGEMENT

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