Full Paper

Synthesis of β-Hydroxypropanoic Acid Derivatives as Potential Anti-inflammatory, Analgesic and Antimicrobial Agents

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A series of new 3-(substituted) 3-hydroxy-propanoic acid ethyl esters **1a–c**, hydrazides **2a–c**, thiosemicarbazides **3a–f**, and semicarbazides **3g**, **3h** has been synthesized. Cyclization of compounds **3a–d** in basic medium yielded 1,2,4-triazole-5-thiones **4a–d**. On the other hand, reaction of hydrazides **2a–c** with CS_2 in basic medium afforded 1,3,4-oxadiazole-5-thiones **5a–c**. All the synthesized compounds were characterized by their physical and spectral analyses data. The newly synthesized compounds were evaluated for their anti-inflammatory, analgesic, and antimicrobial activities. Compounds **1c**, **3g**, **4a**, **4b**, **4c**, and **5c** exhibited comparable anti-inflammatory activity to that of indomethacin and compounds **1c**, **4c**, and **5c** were more analgesics than acetyl salicylic acid. Compounds **4b**, **4c**, and **5c** showed superior GI safety profile (33.3%, 33.3% and 50.0% ulceration) than that of indomethacin (100% ulceration) at 100 mg/kg oral dose. Compounds **4b**, **4c**, and **5c** were also non-toxic with a median lethal dose (LD_{50}) up to 200 mg/kg. The antibacterial and antifungal screenings identified compounds **3c**, **4b**, **4d**, **5a**, and **5b** as the most effective against a variety of tested microorganisms.

Keywords: β-Hydroxypropanoic acid derivatives / Anti-inflammatory / Antimicrobial / 1,2,4-Triazoles / 1,3,4-Oxadiazoles

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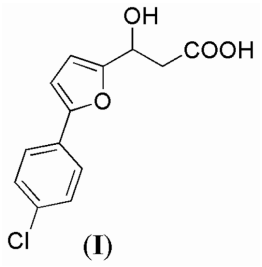
Introduction

Bacterial infections often produce pain and inflammation. In normal practice, two groups of agents (chemotherapeutic, analgesic, and anti-inflammatory) are prescribed simultaneously. Compounds possessing all three activities are not common. Among the many reported classes of anti-inflammatory agents, aryl and heteroaryl (alkyl) carboxylic acids possessed wide clinical applications but with the incidence of gastrointestinal damage and ulceration [1, 2]. β-Hydroxy carboxylic acid derivatives as salicylic acid and its derivatives are well-known drugs for their anti-inflammatory activity [1].



(I)

Figure 1. Chemical structure of F-776 (**I**).

Furthermore, other β-hydroxy carboxylic acid derivatives as F-776 (I) (Fig. 1) and compound **2c** (Scheme 1) are reported to possess a promising anti-inflammatory activity [3, 4]. On the other hand, 1,2,4-triazoles and 1,3,4-oxadiazole derivatives were also reported to possess a variety of pharmacological activities such as anti-inflammatory [5–12], antitubercular [13], antifungal [14], and anticancer activities [15].

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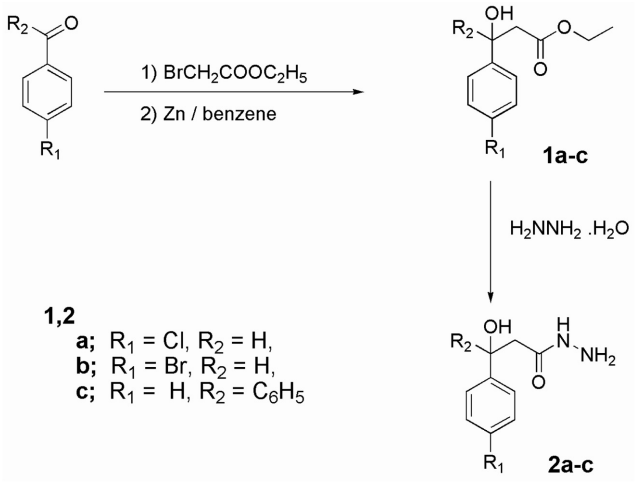


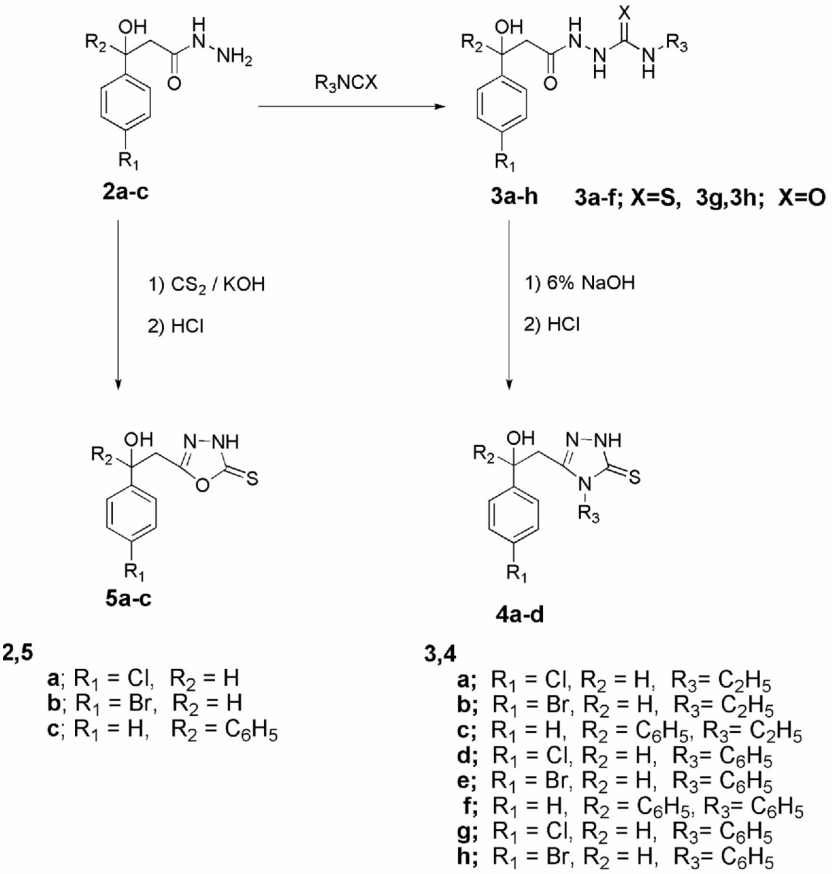
Scheme 1. Synthesis route for compounds **2a–c**.

In view of these facts and in continuation of our search for novel anti-inflammatory agents [16, 17], the present paper involves the design, synthesis, and biological evaluation of a series of 3-(substituted) 3-hydroxypropanoic acid derivatives and their cyclized products.

Results and discussion

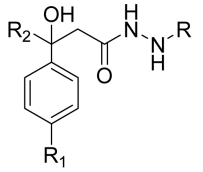
Chemistry

Ethyl 3-(substituted) 3-hydroxypropionates **1a – c** were synthesized starting from the readily available *p*-bromo and *p*-chlorobenzaldehydes or benzophenone via Reformatsky reaction [4, 18, 19]. Hydrazinolysis of the esters **1a – c** afforded the corresponding key intermediate hydrazides **2a – c** (Scheme 1). Reaction of the hydrazides **2a – c** with substituted isothiocyanate and phenyl isocyanate, yielded thiosemicarbazides **3a – f** and semicarbazides **3g** and **3h**, respectively. Cyclization of **3a – d** was carried out through reflux in aqueous NaOH solution affording substituted 1,2,4-triazole-5-thiones **4a – d** in good yields. On the other hand, reaction of the hydrazides **2a – c** with CS_2 in the presence of aqueous NaOH afforded the 1,3,4-oxadiazole-2-thiones **5a – c** (Scheme 2). The structures of the synthesized compounds were verified by IR, ^{1}H-NMR, mass spectra as well as the elemental analyses. Briefly, IR spectra of compounds **2a – c** and **3a-h** showed a strong absorption bands at 3545 – 3140 cm^{-1} (OH, NH, or NH_2 stretching), in addition to a strong absorption band at 1686 – 1638 cm^{-1} for the amidic carbonyl group. Disappearance of the later band upon formation of com-



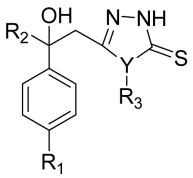
Scheme 2. Synthesis route of compounds **4a – d** and **5a – c**.

Table 1. Physical data of 3-substituted 3-hydroxypropanoic acid derivatives (**2, 3**).



N°	R_1	R_2	R	M. p. (°C)	Yield (%)	Mol. Formula (Mol. Wt.)
2a	Cl	H	H	211-3	80	$C_9H_{11}ClN_2O_2$ (214.65)
2b	Br	H	H	212	80	$C_9H_{11}BrN_2O_2$ (259.10)
2c	H	C_6H_5	H	114-6	95	$C_{15}H_{16}N_2O_2$ (265.30)
3a	Cl	H	$C(S)NHC_2H_5$	170-2	82	$C_{12}H_{16}ClN_3O_2S$ (301.79)
3b	Br	H	$C(S)NHC_2H_5$	164-5	85	$C_{12}H_{16}BrN_3O_2S$ (346.24)
3c	H	C_6H_5	$C(S)NHC_2H_5$	160-2	77	$C_{18}H_{21}N_3O_2S$ (301.79)
3d	Cl	H	$C(S)NHC_6H_5$	158-9	80	$C_{16}H_{16}ClN_3O_2S$ (349.84)
3e	Br	H	$C(S)NHC_6H_5$	183-5	84	$C_{16}H_{16}BrN_3O_2S$ (394.29)
3f	H	C_6H_5	$C(S)NHC_6H_5$	178-80	75	$C_{22}H_{21}N_3O_2S$ (391.49)
3g	Cl	H	$C(O)NHC_6H_5$	200-2	78	$C_{16}H_{16}N_3O_3$ (333.77)
3h	Br	H	$C(O)NHC_6H_5$	200	80	$C_{16}H_{16}BrN_3O_3$ (378.22)

Table 2. Physical data of 1,2,4-triazoles **4a–d** and 1,3,4-oxadiazoles **5a–c**.



N°	R_1	R_2	R_3	Y	M. p. (°C)	Yield (%)	Mol. Formula (Mol. Wt.)
4a	Cl	H	C_2H_5	N	170-2	78	$C_{12}H_{14}ClN_3OS$ (283.78)
4b	Br	H	C_2H_5	N	70-2	80	$C_{12}H_{14}BrN_3OS$ (328.23)
4c	H	C_6H_5	C_2H_5	N	155-8	70	$C_{18}H_{19}N_3OS$ (325.43)
4d	Cl	H	C_6H_5	N	94-6	72	$C_{16}H_{14}ClN_3OS$ (331.82)
5a	Cl	H	–	O	175-7	75	$C_{10}H_9ClN_2O_2S$ (256.71)
5b	Br	H	–	O	175-6	87	$C_{10}H_9BrN_2O_2S$ (301.16)
5c	H	C_6H_5	–	O	155-6	70	$C_{16}H_{14}N_2O_2S$ (298.63)

pounds **4a–d** or **5a–c** is a preliminary indication for the cyclization process. Moreover, ^{1}H-NMR spectra were in accordance with the proposed structures of the synthesized compounds. (Details of structures, yields, melting points, and spectral data are shown in Tables 1 and 2 and in the experimental section, part 3.)

Biological screening

Anti-inflammatory activity

Previously reported results indicated that the conversion of the free acid moiety of NSAIDs into amides resulted in more potent derivatives [20, 21] and some of them showed reduced ulcerogenic effects [22]. Further cyclization of the carboxylic acid hydrazides or thiosemicabazides to azoles such as 1,2,4-triazoles and 1,3,4-oxadiazoles were also reported to yield potent nonulcerogenic derivatives [6, 7].

In the present study, twelve compounds namely **1c**, **2c**, **3f**, **3g**, **3h**, **4a–d**, and **5a–c** were selected to test their anti-inflammatory activity using the carragenan-induced rat paw edema method [23]. Indomethacin was used as a reference drug. All compounds and the reference drug were tested at a dose level of 10 mg/kg and the results are presented in Table 3. After 3 h of administration, compounds **1c**, **3g**, **4a**, **4b**, **4c**, and **5c** exhibited anti-inflammatory activities similar to that of indomethacin while the

Table 3. Effect of compounds **1**, **2**, **3**, **4**, **5**, and indomethacin on carrageenan-induced rat paw edema test (10 mg/kg *i. p.* dose).

Nº	Edema inhibition (%)*				
	0.5 h	**1 h**	**2 h**	**3 h**	**5 h**
Indomethacin	6.7 ± 0.99	12.9 ± 0.79	21.2 ± 0.95	36.4 ± 1.08	44.0 ± 0.83
1c	3.0 ± 0.81	9.7 ± 0.59	30.3 ± 0.75	36.4 ± 0.65	38.2 ± 0.97
2c	3.0 ± 0.78	12.9 ± 0.91	24.2 ± 1.02	24.2 ± 0.82	35.3 ± 0.69
3f	1.0 ± 0.35	6.0 ± 0.49	12.1 ± 0.59	21.1 ± 0.91	17.6 ± 0.87
3g	0.0	9.6 ± 1.27	24.2 ± 1.68	30.3 ± 1.27	35.3 ± 0.90
3h	2.2 ± 0.44	6.5 ± 0.85	15.2 ± 0.66	18.2 ± 1.39	26.5 ± 0.65
4a	3.4 ± 0.83	9.7 ± 0.95	18.2 ± 0.61	30.3 ± 0.86	32.4 ± 0.93
4b	3.4 ± 0.56	16.1 ± 1.37	24.2 ± 0.96	30.3 ± 1.16	38.2 ± 1.31
4c	0.0	9.7 ± 0.31	24.2 ± 0.92	27.3 ± 1.29	41.2 ± 0.78
4d	6.7 ± 0.79	6.5 ± 0.42	12.1 ± 0.98	21.2 ± 0.28	20.6 ± 0.41
5a	0.0	9.7 ± 1.27	24.2 ± 1.33	24.2 ± 1.70	23.5 ± 1.69
5b	3.4 ± 0.86	12.9 ± 0.93	27.3 ± 0.85	24.2 ± 1.02	26.3 ± 0.65
5c	3.4 ± 0.92	12.9 ± 1.36	30.3 ± 1.27	33.4 ± 0.88	38.2 ± 0.84

* Each value represents the mean ± S.E. and all showed at least significant difference at $P < 0.05$ in comparison with control group.

Table 4. Analgesic activity of test compounds in the hot-plate test (10 mg/kg *i. p* dose).

Nº	**Reaction time (s)**[a)]					
	0.5 h	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
Control	19.8 ± 0.45	20.5 ± 0.92	21.1 ± 1.02	22.4 ± 0.61	25.0 ± 0.33	21.1 ± 0.47
Acetyl salicylic acid	36.1 ± 0.92	55.1 ± 1.11	45.4 ± 1.22	36.0 ± 1.02	35.9 ± 1.44	28.6 ± 1.36
1c	37.6 ± 0.81	37.6 ± 0.73	31.6 ± 0.88	30.1 ± 0.72	26.2 ± 0.96	21.3 ± 0.44[b)]
2c	23.0 ± 0.88	33.3 ± 0.46	24.2 ± 1.02	26.0 ± 0.94	20.7 ± 0.56	20.1 ± 0.32
3g	30.3 ± 1.28	32.3 ± 0,98	23.1 ± 0.78	24.5 ± 0.42	25.0 ± 1.11[b)]	23.3 ± 0.99
4b	33.9 ± 0.86	39.1 ± 1.15	41.1 ± 1.36	34.9 ± 1.08	27.3 ± 0.87	25.8 ± 0.45
4c	39.2 ± 1.17	38.5 ± 0.91	39.5 ± 0.13	24.1 ± 1.46	21.6 ± 1.16	22.1 ± 0.95
5c	37.7 ± 1.26	38.9 ± 0.94	39.0 ± 0.83	28.2 ± 1.32	25.9 ± 0.28	25.7 ± 0.86

[a)] Each value represents the mean ± S.E. and all showed at least significant difference at $P < 0.05$ in comparison with control group.
[b)] not significant.

rest of the test compounds gave around half of the activity of indomethacin. After 5 h, compounds **1c**, **2c**, **3g**, **4b**, **4c**, and **5c** exhibited 79 – 93% anti-inflammatory activity compared to that of indomethacin while the rest of the test compounds gave 38 – 72%. As a general result, all cyclized compounds such as **5c** showed an improved anti-inflammatory activity compared to their open analogs as **2c** in accordance with the reported results [6, 7].

Analgesic activity

The most active anti-inflammatory compounds **1c**, **2c**, **3g**, **4b**, **4c**, and **5c** were tested for their analgesic activity relative to acetyl salicylic acid at a dose level of 10 mg/kg [24] and the results are presented in Table 4. At the 3 h interval, the results revealed that the analgesic activity of the test compounds **4b**, **4c**, and **5c** is about 90.4%, 86.9%, and 85.9% of acetyl salicylic acid, respectively. However, compounds **1c**, **4c**, and **5c** were more potent than acetyl salicylic acid at the 5 h interval (104.2%, 108.5%, and 104.3%, respectively) while compound **4b** showed nearly the same activity as that of acetyl salicylic acid (93.8%).

Ulcerogenic effects

The administration of most nonstereoidal anti-inflammatory drugs (NSAIDs) has been limited because of the incidence of gastrointestinal damage (bleeding, ulceration). Compounds **4b**, **4c**, and **5c**, which exhibited both potent an anti-inflammatory and analgesic profile in the pre-mentioned animal models, were evaluated for their ulcerogenic effect in rats [25] and the results are presented in Table 5. The test compounds showed superior GI safety profile (33.3%, 33.3%, and 50.0%) at an oral dose of 100 mg/kg, respectively when compared to indomethacin, which was found to cause 100% ulceration.

Acute toxicity

The median lethal dose (LD_{50}) of the most effective compounds **4b**, **4c**, and **5c** was determined in mice [26] that

Table 5. Gastric ulcerogenic effects of compound **4b**, **4c, 5c**, and indomethacin at 100 mg/kg.

Compound	Ratio of ulcerated animals	% Ulceration	Ulcer index (mean ±S.E.)
Indomethacin	6/6	100	1.01 ± 0.1
4b	2/6	33	1.1 ± 0.2
4c	2/6	33	1.8 ± 0.1
5c	3/6	50	1.0 ± 0.1

Table 6. The antibacterial zones of inhibition (mm) of the test compounds.

Nº	Bacteria				
	Bacillus cereus	*Micrococcus roseus*	*M. loteus*	*E. coli*	*Serrati rodenii*
2a	–	–	–	–	–
2b	–	12	12	–	–
2c	–	–	12	–	–
3a	–	–	–	8	–
3b	–	10	8	–	–
3c	8	16	14	16	14
3d	13	–	15	–	–
3e	8	–	–	8	7
3f	–	–	12	–	–
3g	–	–	–	–	15
4a	14	12	–	15	–
4b	–	–	–	–	17
4c	15	14	16	14	–
4d	–	13	19	–	–
5a	–	12	15	12	12
5b	14	15	14	8	8
5c	–	–	16	–	–
Penicillin G	15	–	10	11	11

were injected (*i.p.*) with graded doses of the test compounds and the LD_{50} was determined. Compounds **4b**, **4c**, and **5c** were also nontoxic with a median lethal dose (LD_{50}) up to 200 mg/kg.

Antimicrobial activities

Antibacterial activity

The newly synthesized compounds (**2a–b**, **3a–g**, **4a–d**, and **5a–c**) were tested for their *in vitro* antibacterial activity against *Bacillus cereus*, *Micrococcus roseus*, and *M. loteus* as representatives of Gram-positive strains and *Escherichia coli* and *Serratia rodenii*, as representatives of Gram-negative ones, using a reported method as described in Experimental (part 3) [27]. The results are cited in Table 6 and expressed as inhibition zones in mm in comparison to penicillin G as a reference drug.

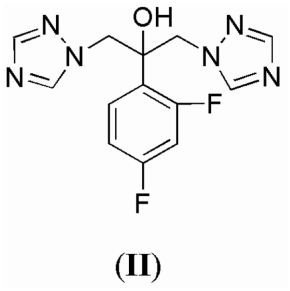
As shown in Table 6, compounds **2a–c** are either inactive or possessed slight activity against one or more of the used microorganisms compared to the reference drug. It is also noted that compound **3c** showed higher activity than penicillin G against all microorganisms. On the other hand, the antibacterial activity was greatly improved when compounds **2a–c** and **3a–c** were cyclized to their corresponding 1,2,4-triazole and 1,3,4-oxadiazole derivatives. Compounds **4a**, **4c**, **4d**, **5a**, and **5b** are more effective than the reference drug against most of the tested microorganisms.

Antifungal activity

Compounds (**2a–b**, **3a–g**, **4a–d**, and **5a–c**) were tested for their *in vitro* antifungal activity using the standard

Table 7. The antifungal zones of inhibition (mm) of the test compounds.

Nº	*Aspergillus*					*Candida albicans*	*Trichophyton*			*Microsporium gypseum*
	A. umigatus	*A. terreus*	*A. flavus*	*A. niger*	*A. ochraceous*		*T. rubrum*	*T. tonsurans*	*T. georgi*	
2a	–	–	–	–	–	–	–	–	–	–
2b	–	–	–	–	–	–	–	–	–	–
2c	–	–	–	–	–	–	–	–	–	20
3a	–	–	–	–	–	–	–	–	–	–
3b	–	–	8	–	–	–	–	–	–	–
3c	7	8	8	12	–	14	14	–	–	20
3d	–	–	7	–	–	–	8	–	–	20
3e	–	–	8	–	–	–	–	–	–	–
3f	–	–	–	–	–	–	8	–	–	–
3g	–	–	–	–	–	–	–	–	–	–
4a	–	–	7	–	–	–	10	–	–	–
4b	10	–	–	–	–	–	–	–	–	13
4c	–	–	–	–	–	–	–	–	–	–
4d	–	7	–	–	–	15	12	12	13	18
5a	10	12	10	14	9	12	12	20	18	25
5b	9	10	13	–	–	8	12	–	–	20
5c	–	–	8	–	–	–	–	–	–	–
Fluconazole	–	–	–	–	–	30	–	25	25	15



(II)

Figure 2. Chemical structure of fluconazole (**II**).

agar disc diffusion method [27] against *Aspergillus fumigatus, A. terreus, A. flavus, A. nige, and A. ochraceous* thom, *Candida albicans* (Robin) Berkhout, *Trichophyton rubrum* (Castellani) Sabouraud, *T. tonsurans*, *T. georgi,* and *Microsporium gypseum* gruby. The results of the antifungal activity are given in Table 7 and expressed as inhibition zones in mm using fluconazole as a reference drug.

Again, compound **2a – c**, **3a – g** (except **3c**) showed slight antifungal activity against most of the used fungi. On the other hand, the antifungal activity was greatly improved with the cyclized 1,2,4-triazole-5-thione and 1,3,4-oxadiazole-2-thione derivatives, which might be attributed to their structural similarity of azoles antifungal agents as fluconazole (**II**) (Fig. 2). Compounds **4d**, **5a**, and **5b** are the most active in this series with compound **5a** showing a broad-spectrum antifungal activity.

Aspergillosis is one of the most serious fungal infections where fluconazole does not appear to be effective in the prevention or treatment of such infections [28]. As shown in Table 7, fluconazole was inactive against all *Aspergillus spp*., while compounds **3b**, **3c**, **3e**, **4a**, **4b**, **4d**, **5a**, **5b**, and **5c** were active against one or more of such species. These results are in accordance with the reported data about the antifungal activity of azoles, which indicate that they are active against dermatophytes such as *Trichophyton*, *Epidermophyton*, *Microsporum spp*., and yeast-like fungi such as *Candida albicans* but are inactive against *Aspergillus spp.* [14, 28]. The difference in the obtained antifungal activity between the tested compounds and fluconazole might be attributed to their different behavior towards the fungal cytochrome P-450, which is responsible for the growth of fungi [14].

Experimental

Melting points were determined on an electrothermal melting point apparatus (Stuart Scientific, Model SMM, UK) and were uncorrected. TLC was carried out using silica gel 60 F_{254} precoated sheets (E. Merck, Darmstadt, Germany) and was visualized using UV lamp (Spectroline Model CM 10, USA), and/or iodine stain.

IR spectra (KBr discs) were recorded on a Shimadzu IR-470 spectrometer (Shimadzu, Japan). ^{1}H-NMR spectra were scanned on a Varian EM-360 L NMR spectrometer (60 MHz), (Varian, USA). Chemical shifts are expressed in δ values (ppm) relative to tetramethylsilane (TMS) as an internal standard, using DMSO-d_6 as solvent. Coupling constants (J values) are expressed in Hertz (Hz). Elemental analyses were performed at the Unit of Microanalysis, Assiut University, Assiut, Egypt. MS spectra were recorded on a JEOL JMS 600 mass spectrometer (JEOL, Japan) at the Microanalytical Center, Faculty of Science, Cairo University, Egypt.

Most of the chemicals used are of commercial grade: *p*-bromobenzaldehyde, *p*-chlorobenzaldehyde, benzophenone, ethyl bromoacetate (Aldrich, Germany), hydrazine hydrate and carboxy methyl cellulose (El Nasr Pharm. Co., Egypt), carrageenan (Sigma, USA), carbon disulphide (Riedel-de Haën), indomethacin (Nile Co., Egypt) and acetyl salicylic acid (ADCO Co., Egypt). Potato dextrose agar (PDA) or sabouraud agar (SA) media are prepared in the Department of Botany, Faculty of Science, Assiut University.

The starting 3-(substituted) 3-hydroxy-propanoic acid ethyl esters (**1a – c**) were prepared via Reformatsky reaction of ethyl bromoacetate with aldehydes or ketones following previously reported literature methods [4, 18, 19].

General method for preparation of 3-(substituted)-3-hydroxy propanoic acid hydrazides (2)

3-(Substituted) 3-hydroxy-propanoic acid ethyl esters **1a – c** (0.01 mol) and hydrazine hydrate (0.02 mol) were refluxed in absolute ethanol (20 mL) for 3 h. The reaction mixture was concentrated, cooled, and poured onto ice-cooled water. The solid thus separated out was filtered, dried, and crystallized from ethanol (Scheme 1, Table 1).

*3-(4-Chlorophenyl)-3-hydroxy propanoic acid hydrazide (**2a**)*

^{1}H-NMR (DMSO-d_6) δ: 2.5 (d, 2H, CH_2, J = 8), 3.6 (brs, 2H, NH_2), 5.0 (t, 1H, CH, J = 8 and 8), 7.2 (s, 4H, Ar-H) 8.5 (s, 1H, NH). IR (KBr) cm^{-1}: 3470, 3300, 3165, 1640, 1610. Anal. calcd. for $C_9H_{11}ClN_2O_2$: C, 50.36; H, 5.17; N, 13.05 Found: C, 50.28; H, 5.46; N, 12.99.

*3-(4-Bromophenyl)-3-hydroxypropanoic acid hydrazide (**2b**)*

^{1}H-NMR (DMSO-d_6) δ: 2.4 (d, 2H, CH_2, J = 8), 3.7 (brs, 2H, NH_2), 5.0 (t, 1H, CH, J = 8 and 7), 7.5 (dd, 4H, Ar-H, J = 10 and 8). IR (KBr) cm^{-1}: 3470, 3295, 3135, 2910, 1638, 1486, 1073, 1010, 820, 734. Anal. calcd. for $C_9H_{11}BrN_2O_2$: C, 41.72; H, 4.28; N, 10.81 Found: C, 41.90; H, 4.47; N, 10.96.

*3-Hydroxy-3,3-diphenylpropanoic acid hydrazide (**2c**) [4]*

^{1}H-NMR (DMSO-d_6) δ: 2.5 (s, 2H, CH_2), 3.4 (brs, 2H, NH_2), 6.8 (s, 1H, OH), 7.2-7.8 (m, 10H, Ar-H), 9.5 (brs, 1H, NH). IR (KBr) cm^{-1}: 3470, 3300, 3165, 1640, 161.

General method for preparation of 3-(substituted) 3-hydroxypropanoic acid thiosemicarbazides (*3a – f*) and semicarbazides (*3g, h*)

A mixture of the corresponding hydrazide **2a – c** (0.01 mol) and the appropriate isothiocyanate or isocyanate derivative (0.01 mol) in ethanol (20 mL) was heated under reflux for 2 – 3 h.

The formed solid was filtered, washed with ethanol, and crystallized from ethanol (Scheme 2, Tables 1).

*1-[3-(4-Chlorophenyl)-3-hydroxypropanoyl]-4-ethylthiosemicarbazide (**3a**)*

^{1}H-NMR (DMSO-d_6) δ: 1.1 (t, 3H, CH_{2CH_3}, J = 8 and 8), 2.5 (d, 2H, CH_2, J = 7), 3.3–3.8 (m, 2H, CH_2CH_3), 4.9–5.3 (m, 1H, CH), 5.9 (d, 1H, OH, J = 8), 7.6 (s, 4H, Ar-H), 7.7 (t, 1H, NH), 9.5 (s, 1H, NH), 10.0 (s, 1H, NH). IR (KBr) cm^{-1}: 3250, 2975, 1686, 1555, 828. Anal. calcd. for $C_{12}H_{16}ClN_3O_2S$: C, 47.76; H, 5.34; N, 13.92. Found: C, 47.89; H, 5.40; N, 13.98.

*1-[3-(4-Bromophenyl)-3-hydroxypropanoyl]-4-ethylthiosemicarbazide (**3b**)*

^{1}H-NMR (DMSO-d_6) δ: 1.2 (t, 3H, CH_{2CH_3}, J = 8 and 8), 2.5 (d, 2H, CH_2, J = 7), 3.3–3.8 (m, 2H, CH_2CH_3), 4.5 (brs, 1H, NH), 5.2 (t, 1H, CH, J = 6 and 6), 5.7 (brs, 1H, OH), 7.4–8.0 (m, 4H, Ar-H), 9.3 (brs, 2H, 2NH). IR (KBr) cm^{-1}: 3435, 3295, 3056, 2945, 1655, 1410, 1180, 1010, 845, 715. Anal. calcd. for $C_{12}H_{16}BrN_3O_2S$: C, 41.63; H, 4.66; N, 12.14. Found: C, 41.90; H, 4.80; N, 11.95.

*1-[3-Hydroxy-3,3-diphenylpropanoyl]-4-ethylthiosemicarbazide (**3c**)*

^{1}H-NMR (DMSO-d_6) δ: 1.1 (t, 3H, CH_3, J = 7 and 7), 3.3–4.0 (m, 4H, $2CH_2$), 6.7 (brs, 1H, OH), 7.8–8.4 (m, 11H, Ar-H and NH), 9.9 (brs, IH, NH), 10.6 (brs, 1H, NH). IR (KBr) cm^{-1}: 3545, 3410, 3190, 2935, 1661, 1636, 1560, 1527, 1215. Anal. calcd. For $C_{18}H_{21}N_3O_2S$: N, 12.23; S, 9.34. Found: N, 12.11; S, 9.23.

*1-[3-(4-Chlorophenyl)-3-hydroxypropanoyl]-4-phenylthiosemicarbazide (**3d**)*

^{1}H-NMR (DMSO-d_6) δ: 2.6 (d, 2H, CH_2, J = 7), 5.0 (t, 1H, CH, J = 8 and 8), 6.1 (brs, 1H, OH), 7.2–7.7 (m, 9H, Ar-H), 9.4, 9.7, 10.1 (3 brss, each of 1H, 3H). IR (KBr) cm^{-1}: 3420, 3265, 1659, 1541, 1472. Anal. calcd. for $C_{16}H_{16}ClN_3O_2S$: C, 54.93; H, 4.61; N, 12.01. Found: C, 55.84; H, 4.89; N, 11.69

*1-[3-(4-Bromophenyl)-3-hydroxypropanoyl]-4-phenylthiosemicarbazide (**3e**)*

^{1}H-NMR (DMSO-d_6) δ: 2.7 (d, 2H, CH_2, J = 7), 5.3 (t, 1H, CH, J = 7 and 7), 6.0 (brs, 1H, OH), 7.3–8.0 (m, 9H, m, Ar-H), 9.7, 10.1, 10.5 (3 brss each of 1H, 3NH). IR (KBr) cm^{-1}: 3420, 3295, 3020, 2910, 1639, 1544, 1487, 1073, 1010, 821, 734. Anal. calcd. for $C_{16}H_{16}BrN_3O_2S$: C, 48.74; H, 4.09; N, 10.66. Found: C, 47.82; H, 4.31; N, 10.24.

*1-[3-Hydroxy-3,3-diphenylpropanoyl]-4-phenylthiosemicarbazide (**3f**)*

^{1}H-NMR (DMSO-d_6) δ: 3.6 (s, 2H, CH_2), 6.8 (brs, 1H, OH), 7.5–8.4 (m, 15H, Ar-H), 10.0 (brs, 1H, NH), 10.5 (brs, 1H, NH), 10.9 (brs, 1H, NH). IR (KBr) cm^{-1}: 3455, 3370, 3285, 3200, 1664, 1636, 1535. Anal. calcd. for $C_{22}H_{21}N_3O_2S$: C, 67.50; H, 5.41; N, 10.73; S, 8.19. Found: C, 67.58; H, 5.53; N, 10.71; S, 8.55.

*1-[3-(4-Chlorophenyl)-3-hydroxypropanoyl]-4-phenylsemicarbazide (**3g**)*

^{1}H-NMR (DMSO-d_6) δ: 2.6 (d, 2H, CH_2, J = 7), 5.0–5.3 (m, 1H, CH), 6.1 (brs, 1H, OH), 7.2–7.7 (m, 9H, Ar-H), 8.3, 8.7, and 9.9 (3 brss, each of 1H, 3H). IR (KBr) cm^{-1}: 3470, 3340, 3215, 1639, 1605, 1590. Anal. calcd. for $C_{16}H_{16}ClN_3O_3$: C, 57.58; H, 4.83; N, 12.59. Found: C, 55.88; H, 5.12; N, 12.67.

*1-[3-(4-Bromophenyl)-3-hydroxypropanoyl]-4-phenylsemicarbazide (**3h**)*

^{1}H-NMR (DMSO-d_6) δ: 2.5 (d, 2H, CH_2, J = 8), 4.3 (brs, 1H, OH), 5.2 (t, 1H, CH, J = 7 and 7), 7.4–8.0 (m, 9H, Ar-H), 8.4, 8.8, 10.1 (3 brss each of 1H, 3NH). IR (KBr) cm^{-1}: 3415, 3225, 3055, 2930, 1674, 1610, 1587, 1483, 1061, 746, 617. Anal. calcd. for $C_{16}H_{16}BrN_3O_3$: C, 50.81; H, 4.26; N, 11.11. Found: C, 51.04; H, 3.68; N, 11.16.

General method for preparation of 3-[2-(substituted)-2-hydroxyethyl]-4-alkyl/aryl-4,5-dihydro-1H-1,2,4-triazole-5-thiones (*4a–d*)

The appropriate thiosemicarbazide, **3a–d** (0.01 mol) was dissolved in 6% aqueous NaOH solution (20 mL) and refluxed for 4 h. The resulting solution was cooled, filtered, and acidified with dilute HCl to pH 5. The solid formed was filtered off, washed with water, dried, and crystallized from aqueous ethanol (Scheme 2, Table 2).

*3-[2-(4-Chlorophenyl)-2-hydroxyethyl]-4-ethyl-4,5-dihydro-1H-1,2,4-triazole-5-thione (**4a**)*

^{1}H-NMR (DMSO-d_6) δ: 1.4 (t, 3H, CH_{2CH_3}, J = 7 and 7), 3.2 (d, 2H, CH_2, J = 8), 3.6 (brs, 1H, NH), 4.0–4.4 (q, 2H, CH_2CH_3), 5.1 (t, 1H, CH, J = 7 and 8), 6.1 (brs, 1H, OH), 7.7–7.8 (m, 4H, Ar-H). IR (KBr) cm^{-1}: 3555, 3415, 3245, 3160, 2970, 1566, 1488. Anal. calcd. for $C_{12}H_{14}ClN_3OS.H_2O$: C, 47.76; H, 5.34; N, 13.92; S, 10.62. Found: C, 48.16; H, 4.81; N, 14.21; S, 10.31.

*3-[2-(4-Chlorophenyl)-2-hydroxyethyl]-4-phenyl-4,5-dihydro-1H-1,2,4-triazole-5-thione (**4b**)*

^{1}H-NMR (DMSO-d_6) δ: 2.9 (d, 2H, CH_2, J = 8), 3.6 (brs, 1H, NH), 4.9 (t, 1H, CH, J = 7 and 7), 5.8 (brs, 1H, OH), and 7.3–7.8 (m, 9H, Ar-H). IR (KBr) cm^{-1}: 3415, 3300, 3190, 2930, 1595, 1494. Anal. calcd. for $C_{16}H_{14}ClN_3OS$: C, 57.91; H, 4.25; N, 12.66; S, 9.66. Found: C, 57.40; H, 4.29; N, 12.26; S, 9.39

*3-[2-(4-Bromophenyl)-2-hydroxyethyl]-4-ethyl-4,5-dihydro-1H-1,2,4-triazole-5-thione (**4c**)*

^{1}H-NMR (DMSO-d_6) δ: 1.2 (t, 3H, CH_{2CH_3}, J = 7 and 7), 3.1 (d, 2H, CH_2, J = 7), 3.5 (brs, 1H, NH), 4.2 (q, 2H, CH_2CH_3), 5.1 (t, 1H, CH, J = 7 and 7), 5.9 (brs, 1H, OH), 7.6 (dd, 4H, Ar-H, J = 9 and 10). IR (KBr) cm^{-1}: 3465, 3045, 2980, 1638, 1490, 1187, 823, and 765. EI-MS: m/z 325 $[M]^{\bullet+}$, 326 $[M+1]^+$, 182. Anal. calcd. for $C_{12}H_{14}BrN_3OS$: C, 43.91; H, 4.30; N, 12.80; S, 9.77. Found: C, 44.04; H, 4.40; N, 12.82; S, 9.63.

*3-[2-(4-Bromophenyl)-2-hydroxyethyl]-4-phenyl-4,5-dihydro-1H-1,2,4-triazole-5-thione (**4d**)*

^{1}H-NMR (DMSO-d_6) δ: 2.8 (d, 2H, CH_2, J = 7), 4.8 (t, 1H, CH, J = 7 and 7), 4.2 (brs, 1H, NH), 5.5 (brs, 1H, OH), 7.1–7.9 (m, 9H, Ar-H). IR (KBr) cm^{-1}: 3415, 3070, 2960, 1564, 1440, 1068, 827, 741. Anal. calcd. for $C_{16}H_{14}BrN_3OS$: C, 51.07; H, 3.75; N, 11.17; S, 8.52. Found: C, 51.04; H, 3.67; N, 11.16; S, 8.53.

General method for preparation of 5-(substituted) 1,3,4-oxadiazole-2(3H)-thiones (*5a – c*)

To a solution of the hydrazides **2a – c** (0.01 mol) in ethanol (15 mL) at 0°C, carbon disulphide (2 mL) and potassium hydroxide (0.6 g) were added. After addition, the reaction mixture was refluxed until the evolution of H_2S ceased. Excess solvents were evaporated under reduced pressure and the residue was dissolved in water and acidified with dilute HCl to pH ~ 5. The precipitate was filtered off, dried, and crystallized from ethanol (Scheme 2, Table 2).

*5-[2-(4-Chlorophenyl)-2-hydroxyethyl]-1,3,4-oxadiazole-2(3H)-thione (**5a**)*

^{1}H-NMR (DMSO-d_6) δ: 3.1 (d, 2H, CH_2, J = 7), 3.5 (brs, 1H, NH), 5.2 (t, 1H, CH, J = 7 and 7), 6.0 (brs, 1H, OH), and 7.6 (s, 4H, Ar-H). IR (KBr) cm^{-1}: 3465, 3370, 1600, 1494. Anal. calcd. For $C_{10}H_9ClN_2O_2S$: C, 46.79; H, 3.53; N, 10.91; S, 12.49. Found: C, 47.77; H, 3.51; N, 10.46; S, 12.97.

*5-[2-(4-Bromophenyl)-2-hydroxyethyl]-1,3,4-oxadiazole-2(3H)-thione (**5b**)*

^{1}H-NMR (DMSO-d_6) δ: 3.1 (d, 2H, CH_2, J = 7), 5.1 (t, 1H, CH, J = 7 and 7), 6.3 (brs, 2H, OH and NH), 7.6 (dd, 4H, Ar-H, J = 13 and 10). IR (KBr) cm^{-1}: 3485, 3050, 2975, 1494, 1180, 824, 768. Anal. calcd. For $C_{10}H_9BrN_2O_2S$: C, 39.88; H, 3.01; N, 9.30. Found: C, 39.35; H, 2.85; N, 9.07.

*5-(2-Hydroxy-2,2-diphenylethyl)-1,3,4-oxadiazole-2(3H)-thione (**5c**)*

^{1}H-NMR (DMSO-d_6) δ: 3.4 (brs, 1H, NH), 3.9 (s, 2H, CH_2), 6.5 (brs, 1H, OH), 7.3 – 7.8 (m, 10H, Ar-H). IR (KBr) cm^{-1}: 3390, 3140, 2995, 2790, 1486. EI-MS: m/z 280 $[M\text{-}H_2O]^{\bullet+}$, 208, 180, 179, 102. Anal. calcd. For $C_{16}H_{14}N_2O_2S$: C, 64.41; H, 4.73; N, 9.39; S, 10.75. Found: C, 65.92; H, 4.71; N, 8.72; S, 10.28.

Biological screening

Animals were housed in separate cages, six animals each, in temperature-controlled rooms at 25°C. Animals were allowed free access to food and water and maintained at 12 h light/dark cycle. The experiments were conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guide lines.

Analgesic and anti-inflammatory activities were performed at the Department of Pharmacology, Faculty of Medicine, Assiut University, Assiut, Egypt.

Bacterial and fungal cultures were obtained from Assiut University Mycological Center (AUMC), and the screening tests were carried out at the Department of Botany, Faculty of Science, Assiut University, Assiut, Egypt.

Anti-inflammatory activity

Compounds **1c**, **2c**, **3f**, **3g**, **3h**, **4a – d**, and **5a – c** were evaluated for their anti-inflammatory activities using the carageenan-induced rat paw edema as previously described [23]. In this procedure, a pedal inflammation in rat paws was induced by sub-plantar injection of 0.2 mL carrageenan (0.2%) suspension into their right hind. Male adult albino rats (100 – 120 g) were divided into 14 groups, of five animals each. The thickness of rat paw was measured by a Veriner caliper (SMIEC, China) before and after 1 h of carrageenan injection to detect the inflammation induced by carrageenan. Test compounds were injected i.p. at doses of 10 mg/kg to the rats 1 h after injection of carageenan. The control group received the vehicle (0.5% NaCMC in water) while the reference group received indomethacin at 10 mg/kg.

The difference between the thicknesses of the two paws was taken as a measure of edema. The measurement was carried out at 0.5, 1, 2, 3, and 5 h after injection of the test compounds, reference drug, and the vehicle. The results of anti-inflammatory activity of the test compounds and the reference drug are listed in Table 3. The percentage edema inhibition was calculated as follows:

$$\%\text{edema inhibition} = (Va - Vb/Va) \times 100$$

where Va is the increase in paw size in the absence of the test compounds or indomethacin and Vb is the increase in paw size after injection of the test compounds or indomethacin

Analgesic activity

The analgesic activity of the most effective anti-inflammatory derivatives, compounds **1c**, **2c**, **3g**, **4b**, **4c**, and **5c** was determined in mice using the hot plate method [24] using acetyl salicylic acid as reference drug. In this method, the latency time taken (seconds) by the mouse to lick its feet or to jump within a Plexiglas cylinder placed on a hot plate surface (55°C) was determined. This reaction time was taken as the end point and the increase in hot plate latency time was taken as a measure of the analgesic activity.

Male adult albino mice (20 – 25 g) were divided into eight groups, each of five animals. Six test compounds and the reference drug were injected *i. p.* at a dose level of 10 mg/kg into mice. The control group of animals was similarly treated with 0.5% NaCMC. The reaction time was evaluated directly after 0.5, 1, 2, 3, 4, and 5 h of injection. The results of the analgesic activity of the test compounds and acetyl salicylic acid are listed in Table 4.

Ulcerogenic effects

Observation of the gastrointestinal mucosa for the presence of lesions following oral administration of a dose of 100 mg kg^{-1} of the test compounds (**4b**, **4c**, and **5c**) as well as indomethacin has been taken as an indication for the ulcerogenic effects. Both the frequency of ulceration (expressed as ratio of ulcerated animals) and the severity of ulceration (expressed as ulcer index) were used for comparison of the tested compound and indomethacin [25]. Four groups each of six male adult albino mice were fasted for 24 h. The tested compounds and indomethacin were administered orally in doses of 100 mg/kg, as suspensions in 0.5% NaCMC aqueous solution. After 6 h, the animals were sacrified, the stomachs were removed and gastric lesions on the mucosa were determined by using stereoscopic microscope. Ulcer was defined as at least one lesion that was 0.5 mm or more in length. All lesions of more than 0.1 mm in length were summed to obtain the ulcer index and results were cited in Table 5. The percentage ulceration for each group was calculated as follows:

$$\%\text{Ulceration} = \frac{\text{number of ulcerated animals in a group}}{\text{total number of animals in the same group}} \times 100$$

Determination of acute toxicity (LD_{50})

The median lethal dose (LD_{50}) of the most effective compounds **4b**, **4c**, and **5c** was determined in mice. Groups of male adult

albino mice, each of five animals (25–30 g) were injected *i.p.* with graded doses of the test compound. The percentage of mortality in each group of animals was determined 72 h after injection.

Antimicrobial screening

Antibacterial activity

Organisms and culture conditions

Five bacterial species representing both Gram-positive and Gram-negative strains were used to test the antibacterial activities of the target compounds: *Bacillus cereus*, *Micrococcus roseus,* and *M. loteus* as representatives of Gram-positive strains and *Escherichia coli* and *Serratia rodenii* as representativs of Gram-negative strains.

Materials and method [27]

A cell suspension of the bacterial strains was prepared from 48 h-old cultures grown on nutrient agar (NA) in sterilized water. One mL of the cell suspension was added to Petri dishes (9 cm diameter) followed by pouring 15 mL of NA into the plates. Plates were shaken gently to homogenize the inoculums. Sterile 5 mm filter paper discs (Whatman) were impregnated with solutions of the tested compound and penicillin G (100 mM/mL in DMSO). In addition, other discs were impregnated with the solvent (DMSO) as a control. The impregnated discs were then dried for 1 h and placed in the center of each plate. The seeded plates were incubated at 35 ± 2°CC for 24–48 h. The radii of inhibition zones (in mm) of triplicate sets were measured and results are given in Table 6.

Antifungal activity

Organisms and culture conditions

Ten pathogenic, phytopathogenic, or food-poisoning fungal species were used in the present study: *Aspergillus fumigatus, A. terreus, A. flavus, A. niger, and A. ochraceous* Thom, *Candida albicans* (Robin) Berkhout, *Trichophyton rubrum* (Castellani) Sabouraud, *T. tonsurans*, *T. georgi,* and *Microsporium gypseum* Gruby.

Materials and method [27]

Spore suspension in sterile water was prepared from 2–5 days-old culture of the test fungi growing on potato dextrose agar (PDA) or sabouraud agar (SA) media. The final spore concentration was 5×10^5 spores/mL. About 15 mL of the growth medium was introduced onto sterilized petri dishes of 9 cm diameter and inoculated with 1 mL of the spore suspension. Plates were shaken gently to homogenize the inoculums. The antifungal activity of the tested compounds was performed by the standard agar disc diffusion method as follows [27]: Sterile 5 mm filter paper discs (Whatman) were impregnated with solutions of the test compounds and fluconazole (100 mM/mL in DMSO). In addition, other discs were impregnated with the solvent (DMSO) and served as a control. The impregnated discs were then dried for 1 h and placed in the center of each plate. The seeded plates were incubated at 28 ± 2°C for 7 days. The radii of inhibition zones (in mm) were measured at successive intervals during the incubation period. Triplicate sets were applied for each treatment and the results are given in Table 7.

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References

[1] R. F. Borne, *Nonsteroidal anti-Inflammatory Agents*, in *Foye's Principles of Medicinal Chemistry*, 5th edition (Eds.: D. A. Williams and T. L. Lemke) Lippincott, Philadelphia **2002**, pp. 751–793.

[2] A. S. Michaelidou, D. Hadjipavlou-Litina, *Chem. Rev.* **2005**, *105*, 3235–3271.

[3] C. T. Huang, K. O. Ellis, *Biochem. Pharmacol*. **1981**, *30*, 3008–3009.

[4] K. Tanaka, K. Matsuo, A. Nakanishi, T. Hatano, *et al.*, *Chem. Pharm. Bull.* **1983**, *31*, 2810–2819.

[5] E. Palaska, G. Sahin, P. Kelicen, N. Tugba Darlu, G. Altinok, *Il Farmaco* **2002**, *57*, 101–107.

[6] M. Amir, S. Kumar, *Arch. Pharm. Chem. Life Sci.* **2005**, *338*, 24–31.

[7] M. Amir, S. Kumar, *Eur. J. Med. Chem.* **2004**, *39*, 535–545.

[8] F. Macaev, G. Rusu, S. Pogrebnoi, A. Gudima, *et al., Bioorg. Med. Chem.* **2005**, *13*, 4842–4850.

[9] S. G. Küçükgüzel, S. Rollas, H. Erdeniz, M. Kiraz, *Eur. J. Med. Chem.* **1999**, *34*, 153–160.

[10] M. M. Burbuliene, V. Jakubkiene, G. Mekuskiene, E. Udrenaite, *et al.*, *Il Farmaco* **2004**, *59*, 767–774.

[11] G. Turan-Zitouni, Z. A. Kaplancikl, M. T. Yildiz, P. Chevallet, D. Kaya, *Eur. J. Med. Chem.* **2005**, *40*, 607–613.

[12] F. A. Omar, N. M. Mahfouz, M. A. Rahman, *Eur. J. Med. Chem.* **1996**, *31*, 819–825.

[13] M. G. Mamolo, D. Zampieri, L. Vio, M. Fermeglia, *et al.*, *Bioorg. Med. Chem.* **2005**, *13*, 3797–3809.

[14] A. R. Martin, *Anti-infective Agents in Wilson and Gisvold's: Textbook of Organic Medicinal and Pharmaceutical Chemistry*, 10th edition, (Eds. J. N. Delgado and W. A. Remers), Lippincott-Raven, Philadelphia, New York **1998**, pp. 185–190.

[15] A. S. Aboraia, H. M. Abdel-Rahman, N. M. Mahfouz, M. A. El-Gendy, *Bioorg. Med. Chem.* **2006**, *14*, 1236–1246.

[16] A. A. Bekhit, H. M. Abdel-Rahman, A. A. Guemei, *Arch. Pharm. Chem. Life Sci.* **2006**, *339*, 81–87.

[17] H. M. Abdel-Rahman, M. M. Sheha, *Medicinal Chemistry: An Indian Journal* **2005**, *1*, 7–13.

[18] P. H. Lee, K. Bang, K. Lee, S.-Y. Sung, S. Chang, *Synth. Commun.* **2001**, *31*, 3781–3789.

[19] A. I. Ayi, R. Condom, P. C. Maria, T. N. Wade, R. Guedj, *Terahedron Lett.* **1978**, *46*, 4507–4510.

[20] A. S. Kalgutkar, S. W. Rowlinson, B. C. Crews, L. J. Marnett, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 521–524.

[21] M. Duflos, M.-R. Nourrisson, J. Brelet, J. Courant, *et al.*, *Eur. J. Med. Chem.* **2001**, *36*, 545 – 553.

[22] H. O. Gulcan, E. Kupeli, S. Unlu, E. Yesilada, M. F. Sahin, *Arch. Pharm. Pharm. Med. Chem.* **2003**, *336*, 477 – 482.

[23] L. V. G. Nargund, G. R. N. Reedy, V. Haripbasad, *J. Pharm. Sci.* **1994**, *83*, 246 – 248.

[24] A. B. Elizabeth, R. M. Frank, E. M. Roger, A. S. Stephen, *et al.*, *J. Med. Chem*. **1986**, *29*, 894 – 897.

[25] B. Tozkoparan, G. Akaty, E. Yesilada, *Il Farmaco* **2002**, *57*, 154 – 152.

[26] F. Sztaricskai, I. E. Takacs, F. Pusztai, G. Szabo, I. Csipo, *Arch. Pharm. Pharm. Med. Chem*. **1999**, *332*, 321 – 326.

[27] H. William, *Microbiological assay, An Introduction to Quantitative Principles and Evaluation*, Academic press, New York, **1977**.

[28] J. E. Benett, *Antimicrobial agents* in *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 9th edition (Eds. P. B. Molinoff and R. W. Rudden) McGraw-Hill, New York **1996**, pp. 1175 – 1190.

