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QSAR Studies of some New Synthesized Diacylhydrazine Compounds Derived from Indomethacin as Caspase-1 Inhibitors

Kawther H. Al-Naimi Haitham A. A-Alwahb Hyffaa Y. Hussien
Department of Chemistry/ College of Science/ University of Mosul

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ABSTRACT

Indomethacin (1) reacts with absolute ethanol in presence of conc. sulphuric acid to form the ester (2). This ester was converted into the hydrazide (3) by treatment with hydrazine hydrate. The reaction of the hydrazide (3) with acid chlorides (of indomethacine, dichlofenac, mefenamic acid, aspirin and ibuprofen) in dry xylene afforded the *N,N'*-diacylhydrazine compounds (4-8). A series of new 2,5-disubstituted-1,3,4-oxadiazoles (9-13) derived from indomethacin (1) was obtained by the dehydrative cyclization of the corresponding *N,N'*-diacylhydrazines(4-8), in presence of an excess of thionyl chloride. The quantitative structure-activity relationship (QSAR) analysis of the synthesized compounds was also performed to compute the parameters that affect the biological activity (inhibitory activity IC_{50}). Docking analysis was also performed to predict the interactions between the synthesized compounds with Caspase-1 (Interleukin-1 converting enzyme). The study is aimed to predict factor affecting the biological activity of the prepared compounds by employing the QSAR analysis. The structures of the synthesized compounds were confirmed by the spectroscopic methods.

Keywords: Indomethacin, *N*, *N'*-diacylhydrazine, 2,5-disubstituted-1,3,4-oxadiazoles, QSAR and docking.

دراسة QSAR لعدد من مركبات ثنائي الاسيل هيدرازين الجديدة المشتقة من الإندوميثاسين بوصفها مثبطات لـ Caspase-1

الملخص

تفاعل الإندوميثاسين (1) مع الإيثانول المطلق بوجود حامض الكبريتيك المركز لتكوين الأستر (2) الـذي حـول إلـى الهيدرازيد (3) بمعاملته مع الهيدرازين المائي. أما تفاعل المركب (3) مع كلوريدات الحوامض (الإندوميثاسين، الدايكلوفيناك، حامض الميفينامك، الأسبرين، الايبوبروفين) فإنه أعطى مركبات الثنائي أسيل هيدرازين (4-8). حـضرت مركبـات 5،2- ثنائي التعويض-1،3،4-أوكسادايازول (9-13) بحولقة المركبات 1،2- ثنائي أسيل هيدرازين (4-8) المقابلة باستخدام زيـادة من كلوريد الثايونيل بوصفه عامل حولقة وساحباً لجزيئة الماء. تضمن هذا البحث أيضاً دراسة وتحليل العلاقـة الكميـة بـين التركيب والفعالية QSAR واستنباط أنموذج رياضي يربط الفعالية الحيوية لهذه المركبات بوصفها مثبطات لإنزيم Caspase-1 وحساب العوامل المؤثرة عليها، وتحليل الإرساء لحساب طاقة التجاذب بين هذه المركبات والإنزيم الهدف المتوقع وهو إنـزيم Caspase-1. شخصت تراكيب المركبات المحضرة بوساطة الطرق الطيفية.

الكلمات الدالة: إندوميثاسين، *N',N*- ثنائي أسيل هيدرازين، مركبات 5،2- ثنائي التعويض 1،3،4-أوكـسادايازول، QSAR والـ Docking.

INTRODUCTION

The 2,5-disubstituted-1,3,4-oxadiazole compounds have been reported to be remarkable anti-depressive (Almasirad *et al*., 2004), anticonvulsive (Kashaw *et al*., 2010), antihypertensive (Bankar *et al*., 2004), anti-inflammatory (Chandra *et al*., 2010), antiviral (Wang *et al*., 2012), antitumor (Savariz *et al*., 2010), antimicrobial agents (Oliveira *et al*., 2012), as well as insecticides (Shi *et al*., 2001). Moreover, they have an interesting electrochemical (Yasuda *et al*., 2005) and fluorescent properties (Agbaria and Gill, 1994). *N,N'*-diacylhydrazines are easily accessible starting from acids, acid chlorides or esters and respectively various hydrazides (Dingemans *et al*., 2001) and are valuable intermediates in the synthesis of cyclic compounds. The most common synthetic strategy for preparing 2,5-substituted-1,3,4-oxadiazoles involves the dehydrative cyclization of *N,N'*-diacylhydrazines using dehydration agents such as $POCl_3$ (Amir and Kumar, 2007), $SOCl_2$ (Hernández-Ainsa *et al*., 2012) or P_2O_5 (Carlsen and Jorgensen, 1994). The present work is concerned with the preparation of compounds from *N,N'*-diacylhydrazines containing indomethacin moiety and studying its biological activity as Caspase-1 inhibitor by using quantitative structure activity relationship (QSAR). On the other hand, the QSAR is considered one of the most important field in medicinal chemistry, giving useful information applied to drug design. QSAR models are mathematical equations relating chemical structure to a wide variety of physical, chemical and biological properties. The derived relationship between molecular descriptors and activity is used to estimate the property of other molecules and/or to find the parameters affecting the biological activity (Hansch, 1969).

EXPERIMENTAL

Melting points were determined on an electrothermal IA 9300 Digital-series (1998) apparatus, and they were uncorrected. Infrared spectra were recorded on a Bruker FT-IR spectrophotometer Tensor 27, Germany (College of Education, Mosul University). UV spectra were recorded on a Shimadzu UV/Vis–1650 pc spectrophotometer using chloroform as a solvent (College of Science, Mosul University).

The QSAR study was performed using the previously reported biological activity values (IC_{50} nM) (Soper *et al*., 2006). These values are used as dependent variables. The physicochemical properties [i.e. Lipophilic (CLogP) and Electrostatic (Chemical hardness η)] were calculated using AM_1 as an independent variable in linear regression analysis, then the QSAR models were designed using SPSS (11.5) program. The docking process was carried out using iGEMDOCK v.2.1 program.

Synthesis of ethyl [1-(4-chlorobenzoyl)-2-methyl-5-methoxy-1H-indol-3-yl] acetate (2). General method (Euranto, 1969)**:**

To a solution of Indomethacin (1) (0.1 mole, 35.75 g) in 100 ml absolute ethanol, 10 ml of conc. sulfuric acid was slowly added at room temperature. The reaction mixture was refluxed for 6 hr. The contents of the flask were allowed to cool to room temperature, then poured onto crushed ice and neutralized with 20% sodium bicarbonate solution. The slurry oil was extracted with benzene. The organic layer was dried over anhydrous magnesium sulphate, filtered off and evaporated under reduced pressure to give semi solid product, which recrystallized from n-heptane to give white bright needle crystals; m.p 70-72 ^oC in 90% yield. ^{1}H-NMR (200 MHz, DMSO-d6) δ ppm: 1.15 (t, CH_2-CH_3), 4.08 (q, CH_2-CH_3), 2.3 (s, CH_3), 3.6 (s, CH_2), 3.75 (s, OCH_3), 6.6-7.15 (m, 7H, Ar-H). IR (KBr pellets) ν cm^{-1}: 1711 (C=O str., ester), 1626 (C=O str., ter. amide), 1589 (C=C str.), 3003 (C-H str., aromatic), 2976 (C-H str., aliphatic), 793 (C-Cl str.). U.V ($CHCl_3$) λ nm: 284 ($n \rightarrow \pi^*$), 250 ($\pi \rightarrow \pi^*$) electronic transition.

Synthesis of [1-(4-chlorobenzoyl)-2-methyl-5-methoxy-1H-indol-3-yl] acetic acid hydrazide (3).

General method (Amir and Shikha, 2004):

To a solution of ester (2) (0.1 mole, 38.55 g) in 100 ml absolute ethanol, hydrazine hydrate (0.5 mole, 7.26 ml) (the hydrazine hydrate (80%) was freshly distilled in presence of sodium hydroxide) was added and the reaction mixture was refluxed for 48 hr. The precipitate was separated on cooling and collected by filtration, then recrystallized from dry benzene to afford the hydrazide (3) as white bright crystals; m.p 161-163 ^{o}C in 93% yield. ^{1}H-NMR (200 MHz, DMSO-d6) δ ppm: 2.2 (s, CH_3), 3.4 (s, CH_2), 3.75 (s, OCH_3), 4.35 (br, NH_2), 6.6-7.15 (m, 7H, Ar-H), 10.58 (s, NH, amide). IR (KBr disk) ν cm^{-1}: 1654 (C=O str., amide), 1631 (C=O str., ter. amide), 1594 (C C str.), 3282 (N-H str., amide), 3300 (NH_2 str.), 3014 (C-H str., aromatic), 2907 (C-H str., aliphatic), 794 (C-Cl str.). U.V ($CHCl_3$) λ nm: 278 ($n \rightarrow \pi^*$), 246 ($\pi \rightarrow \pi^*$).

Synthesis of diacylhydrazine compounds (4-8) (Al-Hamdany, 2009)**:**

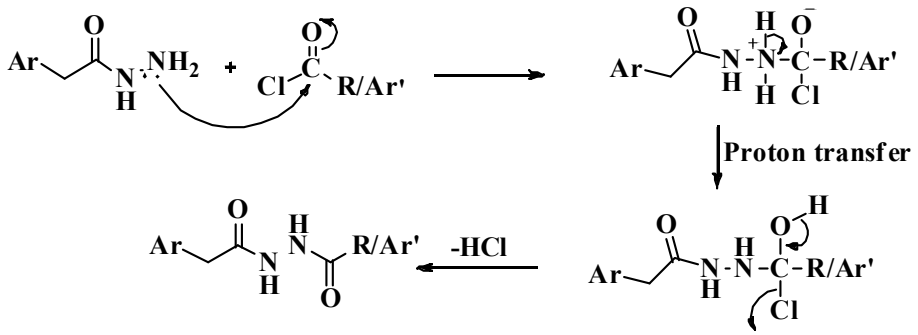
To a solution of an acid (indomethacine, dichlofenac, mefenamic acid, aspirin or ibuprofen) (0.01 mole) in 20 ml of dry benzene, (0.013 mole, 1.53 g) of thionyl chloride was slowly added then the reaction mixture refluxed for 4 hr. The mixture (benzene and excess of thionyl chloride) was evaporated under reduced pressure to dryness. To the solid residue, a solution of acylhydrazine (3) (0.015 mole,5.565 g) in 40 ml of dry xylene and (0.01 mole, 0.82 ml) dry pyridine was added. The reaction mixture was stirred under reflux for 60 min. The resulted precipitate was filtered off and washed with water, dried and recrystallized from a mixture of ethyl acetate and ether or petroleum ether (80-100) ^{o}C. The physical properties and spectral data of compounds (4-8) were listed in (Table 1).

Synthesis of the oxadiazoles (9-13) (Kumar *et al*., 2010):

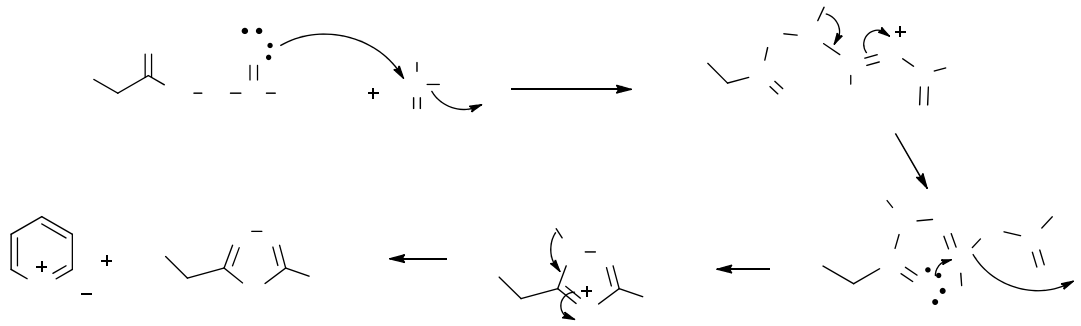
To a suspension of (0.01 mole) of *N*,*N*'-diacylhydrazine (4-8) in (5 ml) thionyl chloride, (0.5 ml) dry pyridine were added dropwise. The mixture was stirred at room temperature overnight, then refluxed for 30 min. The pyridine and the remained thionyl chloride were removed under vacuum. The remained residue was recrystallized from ethanol to give pure products (9-13). The physical properties and spectral data of compounds (9-13) were listed in (Table 1).

RESULTS AND DISCUSSION

Ethyl [1-(4-chlorobenzoyl)-2-methyl-5-methoxy -1H-indol-3-yl] acetate (2) was obtained via Buchman synthesis using indomethacin in presence of concentrated sulphuric acid as catalyst in ethanolic solution (Euranto, 1969). The α-(3-indomethacin) acetyl hydrazine (3) was synthesized by condensation of indomethacin ester (compound 2) with hydrazine hydrate in absolute ethanol. The IR spectrum of the hydrazide (3) showed the disappearance of carbonyl ester absorption band at (1711cm^{-1}) and appearance of strong absorption band at (1654cm^{-1}) and medium absorption band at (1631cm^{-1}) due to the carbonyl hydrazide and tertiary carbonyl amide, respectively. The key intermediates for synthesis of 2,5-disubstituted-1,3,4-oxadiazoles (9-13) are the *N*,*N*'-diacylhydrazines (4-8), which were formed as a result of the reaction of the acid chloride of (indomethacine, dichlofenac, mefenamic acid, aspirin or ibuprofen) and the hydrazide **3**, in dry xylene across the tetrahedral mechanism:



The 2,5-disubstituted-1,3,4-oxadiazoles was synthesized by refluxing of *N,N'*-diacylhydrazines with an excess of $SOCl_2$ in dry xylene, according to following suggested mechanism:



The structures of compounds (9-13) were confirmed by physical and spectral data. The I.R. spectrum showed the new absorption bands at (1606-1620 cm^{-1}) due to the (C=N) bond stretching.

The QSAR study was performed using the calculation of lipophilicity (Clog*P*) and Electrostatic (Chemical hardness η) as shown in (Table 2) as independent variables in Linear regression analysis which give a QSAR models as shown in equation (1) for 13 compounds described in (Table 2) to obtain inhibitory activity IC_{50} toward Caspase-1 (Interleukin-1 converting enzyme) the target is Homo sapiens.

$$IC_{50}= -3886.37+ 94.18*CLogP + 911.00*\eta \quad \ldots\ldots(1)$$

(n =13; correlation coefficient (*r)* = 0.913)

η = (E LUMO – E HOMO)/2

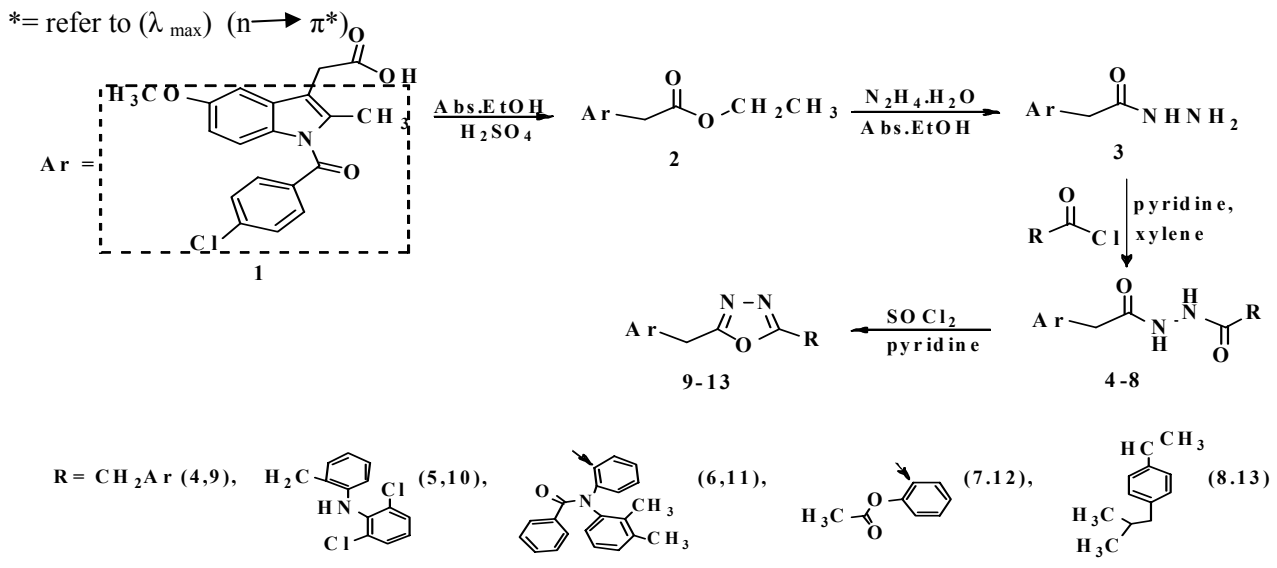
The best biological activity of the synthesized compounds as Caspase-1 inhibitor was exhibited by compound 7 as shown in Table (3), while for the proposed compounds (14-23), the best biological activity as Caspase-1 inhibitor was exhibited by compound 17 as shown in Table (5).

Molecular docking studies have been carried out to get an insight into the inhibitor of Caspase-1 by the selective antagonist of indomethacin derivative compounds. Our investigation has shown that the best docking energy is -138.991 kcal/mol for the synthetic compound (4) with Caspase-1 as shown in Table (4) (VDW: Vander Waals, H bond: Hydrogen bond.

Table 1: The physical properties and spectral data for compounds 4-13.

Comp. No.	Colour	m.p °C	Yield %	U.V ($CHCl_3$) λ nm	IR (KBr) ν cm^{-1}			
					C=O sec.amide	C=N	C=O ter.amide C=C	C-Cl N-H
4	white	301-303	75	218 248*	1685		1601 1466-1597	756 3186
5	Reddish-orange	311-313	28	246 268*	1690		1601 1471-1560	742 3201
6	Pink	258-260	37	220 256*	1680		1637 1458-1558	756 3238
7	Yellow	241-243	15	222 250*	1684 1735(ester)		1637 1457-1600	762 3236
8	white	298-300	17	225 244*	1685		1647 1466-1601	742 3221
9	white	251-253	60	239 302*		1606	1637 1458-1554	739
10	Pale-pink	248-250	94	244 294*		1616	1643 1489-1598	748 3240
11	Brown	177-180	86	222 246*		1606	1675 1466-1575	748
12	gray	145-148	78	246 282*	1757/ester	1620	1647 1466-1598	748
13	white	252-254	80	225 246*		1610	1647 1487-1575	754

= refer to (λ_{max}) (n → π)



Scheme 1: Synthesis of some *N,N'*-diacylhydrazines Linked to 2,5-disubstituted-1,3,4-oxadiazoles.

Table 2: Biological activity data of compounds reported in literature (Soper *et al*., 2006) and physicochemical properties

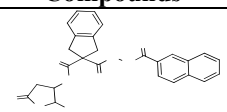
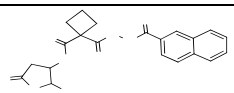
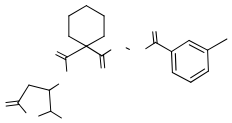
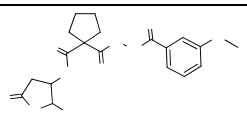
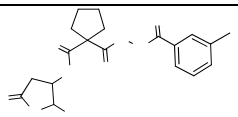
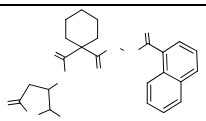
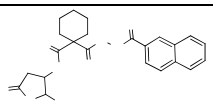
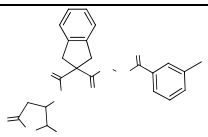
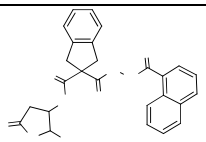
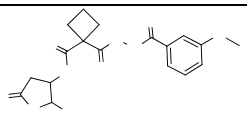
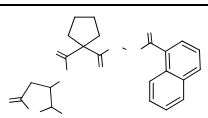
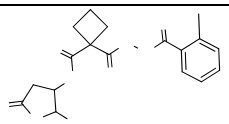
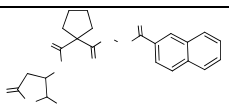
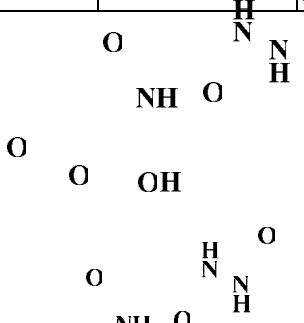
Compounds	IC_{50} nM	CLogP	η e.v
	30	2.141	4.1457
	50	1.222	4.137
	430	1.665	4.498
	200	0.808	4.508
	490	1.106	4.547
	50	2.340	4.135
	90	2.340	4.162
	430	1.466	4.584
	40	2.141	4.129
	260	0.249	4.518
	40	1.781	4.113
	310	0.207	4.672
	150	1.781	4.140
			

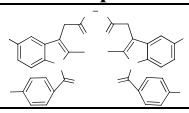
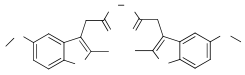
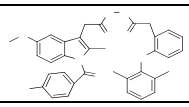
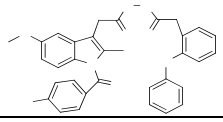
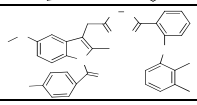
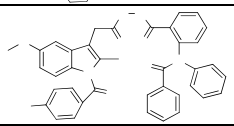
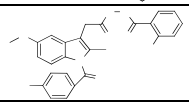
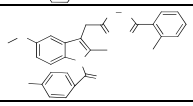
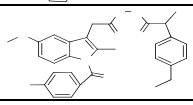
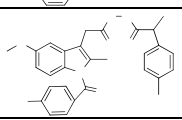
Table 3: Biological activity and physicochemical data as Caspase-1 inhibitors

Compds.	Mol.Wt.	CLog P	η e.v	IC_{50} nM
4	711.59	7.789	3.774	285.513
5	649.95	8.336	3.743	308.522
6	699.19	8.426	3.853	417.400
7	533.96	4.406	3.861	46.230
8	546.06	6.703	3.878	277.435

Table 4: Docking analysis for synthetic compounds

Compds.	Energy kcal/mol	VDW kcal/mol	H.Bond kcal/mol
4	-138.991	-128.923	-10.068
5	-117.770	-107.363	-10.407
6	-118.609	-116.421	-2.188
7	-100.457	-71.567	-28.890
8	-104.559	-94.028	-10.531

Table 5: Biological activity and physicochemical data of proposed compounds

No.	Compds.	Mol.Wt.	CLog P	η e.v	IC_{50} nM
14		683.54	6.618	3.865	258.274
15		434.49	3.178	4.139	183.430
16		609.11	7.500	3.746	232.266
17		581.06	6.502	3.640	41.596
18		595.09	8.016	3.856	381.261
19		671.14	7.878	3.830	344.715
20		491.92	5.467	3.868	152.352
21		489.95	5.004	3.871	111.226
22		532.03	6.361	3.865	234.069
23		518.00	5.832	3.831	153.261

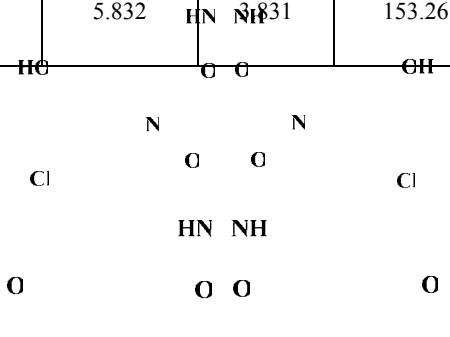


Table 6: Docking analysis for proposed compounds

No.	Energy kcal/mol	VDW kcal/mol	H.Bond kcal/mol
14	-116.103	-94.747	-21.357
15	-99.450	-78.147	-21.303
16	-103.660	-93.419	-10.241
17	-119.742	-105.742	-14.000
18	-122.261	-119.148	-3.113
19	-124.683	-116.799	-7.884
20	-94.983	-76.044	-18.939
21	-105.264	-99.264	-6.000
22	-109.420	-101.950	-7.470
23	-107.346	-101.124	-6.222

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