A STUDY OF H-BOND IN A GROUP OF NSAID HYDROXAMIC ACID DERIVATIVES BY FTIR AND NMR SPECTROSCOPY

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INTRODUCTION

The investigation of drug properties (i.e. chemical structure, physico-chemical properties, the possibility of H-bonding and drug molecular geometry) on which pharmacological effect, metabolism, drug biotransformation pathways and structure of formed metabolites is based on, are of the toxycological, pharmacological and biomedical interest.

The growing interest in the synthesis of therapeutics based on the hydroxamic functional group(s) is observed, although the structures of hydroxamic acids are still the subject of many controversies, among others due to the possibility of keto-iminol tautomerism. On the other hand, hydroxamic acids can exist in the form of *cis* (Z) - and/or *trans* (E)-conformers (relative to C-N bond) which are stabilized by intra- or intermolecular hydrogen bonds. The conformational behaviour of a series of monohydroxamic acids, derivatives of NSAIDs (ibuprofen, fenoprofen, ketoprofen, diclophenac and indomethacin) in DMSO solution and in the solid state has been investigated using FTIR and one- and two-dimensional homo and heteronuclear ^1H and ^{13}C NMR spectroscopy. Hydroxamic acids were synthesized in the reaction of appropriate reactive NSAID benzotriazolide with hydroxylamine hydrochloride or with benzyloxyamine hydrochloride following the reduction. The results of IR and NMR investigations showed significant hydrogen bonding effects in both, the solid state and solution.

NMR spectra (^1H and ^{13}C, COSY, NOESY, HMBC, HETCOR) of NSAID hydroxamic acids measured in DMSO-d_6 solution have showed that investigated NSAID hydroxamic acids in DMSO solution are in the keto form. ^1H NMR chemical shifts of hydroxamic OH are in the range from 10.12 to 11.47 ppm, and hydroxamic NH from 8.61 to 8.97 , while ^{13}C NMR shifts for hydroxamic C=O are in the range from 167.81 to 170.52 ppm. Findings from NMR analysis are in good agreement with FTIR spectral data of solids (KBr).

MATERIAL AND METHODS

The investigated compounds were synthesized from NSAID in the reaction of appropriate NSAID benzotrazolide with substituted hydroxylamines, following the reduction of resulted intermediates (IM) with H_2/Pd.

FTIR spectroscopy

The IR spectra were recorded on a FT-IR Paragon 500 spectrophotometer (Perkin Elmer) from KBr pelleted sample.

NMR spectroscopy

The one- and two-dimensional homo- and heteronuclear ^1H and ^{13}C NMR spectra were recorded with a Bruker AV-600 spectrometer, operating at 600.133 MHz for the ^1H nucleus and 150.917 MHz for the 13C nucleus. Samples were measured from DMSO-d_6 solutions at 27 °C (300 K) in 5 mm NMR tubes. Chemical shifts, in ppm, are referred to TMS as internal standard. FID resolution in ^1H NMR and ^{13}C NMR spectra was 0.29 Hz and 0.54 Hz per point, respectively. The following measurement techniques were used: standard ^1H, APT, COSY, NOESY, HMQC and HMBC. The COSY with standard š/2 pulse sequence was measured using 2048 points in F2 dimension and 512 increments in F1 dimension. Increments were obtained by 4 scans each, 8012.82 Hz spectral width and a relaxation delay of 1.0 s. The FID resolution was 3.91 Hz/point and 15.65 Hz/point in F2 and F1 dimensions, respectively. The NOESY spectra were measured in phase-sensitive mode, with mixing time of 0.50 s and 16 scans per each increment. The spectral width was 6127.45 Hz, 2048 points in F2 dimension and 512 increments in F1 dimension, subsequently zero-filled to 1024 points. The resulting FID resolution was 2.99 Hz/point and 11.96 Hz/point in F2 and F1 dimensions, respectively. The HMQC spectra ($^1J_{C,H}$ was set to 145 Hz) were recorded with 1024 points in F2 dimension and 256 increments in F1 dimension, subsequently zero-filled to 1024 points. For each increment 32 scans were collected, using relaxation delay of 1.0 s. The spectral widths were 6067.96 Hz (F2) and 25000 Hz (F1), with corresponding resolution of 2.96 and 97.65 Hz/point in F2 and F1 dimensions, respectively. The HMBC spectra were measured with 1024 points and 8012.82 Hz spectral width in F2 dimension and relaxation delay of 1.0 s. The additional delay of 0.065 s was used for detecting the long-range C, H couplings. The spectral width in F1 dimension was 33560 Hz, while 256 increments were recorded, each by 32 scans. The FID resolution was 3.912 and 131.08 Hz per point in F2 and F1 dimensions, respectively. The 2D NMR spectra, except NOESY, were measured in pulsed field gradient mode (z-gradient).

RESULTS and DISCUSSION

Table 1. Characteristic stretching vibrations, ν (cm^{-1}), recorded in KBr of synthesized NSAID hydroxamic acids (HA) and their intermediates (IM)

Compound	IR (KBr) ν_{max} (cm^{-1})			
	O-H	N-H	C=O	C=C-H
Ibuprofen HA	3191		1634	3025
Ibuprofen IM	-	3424 3156	1674 1648	3068 3028
Fenoprofen HA	3426	3215	1628	3038
Fenoprofen IM	-	3181	1658	3065 3033
Indomethacin HA	3243		1652 1635	3015
Indomethacin IM	-	3240	1685 1657	3090 3070 3033
Benzylprofen HA	3214		1629	3035
Ketoprofen IM	-	3188	1660	3088 3063 3031
Diclofenac HA	3289	3235	1660	3031
Diclofenac IM	-	3265 3153	1655 1636	3065
N1-Me-diclofenac	3272		1614	3066 3036

Table 2. ^1H chemical shifts (δ/ppm) and H-H coupling constants ($^nJ_{HH}$/Hz)[a] of synthesized NSAID hydroxamic acids (HA)

	Ibuprofen HA	Fenoprofen HA	Indomethacin HA	Benzylprofen HA	Diclofenac-HA	*N*1-Me-Diclofenac HA
H-2	3.40 (1H) ^3J=7.02 (q)	3.43 (1H) ^3J=7.02 (q)	3.40 (2H) (s)	3.40 (1H) ^3J=6.83 (q)	3.47 (2H) (s)	3.87 (2H) (s)
H-3	1.32 (3H) ^3J=7.00 (d)	1.31 (3H) ^3J=7.02 (d)		1.31 (3H) ^3J=6.95 (d)		
H-5		7.00 (1H) (s)	7.15 (1H) (s)	7.20-7.24 (1H) (m)	6.31 (1H) ^3J=7.91 (d)	6.30 (1H) ^3J=7.87 (d)
H-5,6	7.22 (2H) ^3J=7.96 (d)					
H-6					6.86 (1H) ^3J=7.14 (t)	6.86 (1H) ^3J=7.20 (t)
H-7		7.10 (1H) ^3J=7.68 (d)	6.71 (1H) ^3J=8.83 (d)		7.04 (1H) ^3J=7.17 (t)	7.05 (1H) ^3J=7.23 (t)
H-8		7.30 (1H) ^3J=7.89 (t)	6.92 (1H) ^3J=8.87 (d)		7.17 (1H) ^3J=6.97 (d)	7.20 (1H) ^3J=7.03 (d)
H-7,8				7.14-7.20 (2H) (m)		
H-8,9	7.07 (2H) ^3J=7.94 (d)					
H-9		6.84 (1H) ^3J=8.10 (d)		7.06 (1H) ^3J=7.22 (d)		3.17 (3H) (s)
H-7,9						
H-11			3.77 (3H) (s)			
H-12			2.26 (3H) (s)			
H-1'	3.40 (2H) ^3J=7.09 (d)			3.91 (2H) (s)		
H-2'	1.79 (1H) ^3J=6.71 (heptet)					
H-2', H-6'		7.00 (2H) ^3J=7.70 (d)				
H-3', H-5'		7.39 (2H) ^3J=8.49 (t)			7.50 (2H) ^3J=8.00 (d)	7.51 (2H) ^3J=8.05 (d)
H-2', H-4'	0.85 (6H) ^3J=6.55 (d)					
H-4'		7.14 (1H) ^3J=7.37 (t)			7.15 (1H) ^3J=8.08 (t)	7.16 (1H) ^3J=8.05 (t)
H-4', H-6'			7.64 (2H) ^3J=8.00 (d)	7.20-7.24 (2H) (m)		
H-5'				7.20-7.24 (1H) (m)		
H-3', H-7'			7.68 (2H) ^3J=7.85 (d)	7.25-7.30 (2H) (m)		
H-1*						
H-3*-H-7*						
NH1 NH2	8.76 (1H) (bs)	8.80 (1H) (bs)	8.84 (1H) (bs)	8.78 (1H) (bs)	10.92 (1H) (bs) 8.50 (1H) (bs)	7.84 (1H) (bs)
OH	10.60 (1H) (bs)	10.62 (1H) (bs)	10.66 (1H) (bs)	10.62 (1H) (bs)	9.02 (1H) (bs)	10.32 (1H) (bs)

[a] n denotes number of chemical bonds between nuclei through spin-spin coupling occur; (s) singlet, (d) doublet, (t) triplet, (q) quartet, (heptet) heptet

Table 3. ^1H chemical shifts (δ/ppm) and H-H coupling constants ($^nJ_{HH}$/Hz)[a] of synthesized NSAID hydroxamic acid intermediates (IM).

	Ibuprofen IM	Fenoprofen IM	Indomethacin IM	Ketoprofen IM	Diclofenac IM
H-2	3.38 (1H) ^3J=7.04, (q)	3.40 (1H) ^3J=6.65, (q)	3.40 (2H) (s)	3.56 (1H) ^3J=7.00, (q)	3.49 (2H) (s)
H-3	1.31 (3H) ^3J=6.95 (d)	1.31 (3H) ^3J=7.00 (d)		1.39 (3H) ^3J=7.0 (d)	
H-5		6.97 (1H) (s)	7.13 (1H) (s)	7.72 (1H) (s)	6.32 (1H) ^3J=7.95 (d)
H-5,6	7.19 (2H) ^3J=7.64 (d)				
H-6					6.87 (1H) ^3J=7.34 (t)
H-7		7.07 (1H) ^3J=7.67 (d)	6.72 (1H) ^3J=8.89 (d)		7.06 (1H) ^3J=7.54 (t)
H-8		7.28-7.31 (1H) (m)	6.94 (1H) ^3J=8.94 (d)	7.69 (1H) ^3J=7.39 (t)	7.18 (1H) ^3J=7.1 (d)
H-7,8					
H-8,9	7.09 (2H) ^3J=7.69 (d)				
H-9		6.87 (1H) ^3J=8.01 (d)			
H-7,9				7.62 (2H) ^3J=7.13 (d)	
H-11			3.76 (3H) (s)		
H-12			2.23 (3H) (s)		
H-1'	2.41 (2H) ^3J=7.03 (d)				
H-2'	1.81 (1H) ^3J=6.66 (heptet)				
H-2', H-6'		7.01 (2H) ^3J=7.76 (d)			
H-3', H-5'		7.39 (2H) ^3J=8.34 (t)			7.52 (2H) ^3J=8.05 (d)
H-3', H-4'	0.85 (6H) ^3J=6.49 (d)				
H-4'		7.14 (1H) ^3J=7.36 (t)			7.16 (1H) ^3J=7.94 (t)
H-4', H-6'			7.65 (2H) ^3J=8.32 (d)	7.57 (2H) ^3J=7.63 (t)	
H-5'				7.52 (1H) ^3J=7.62 (t)	
H-3', H-7'			7.69 (2H) ^3J=8.35 (d)	7.75 (2H) ^3J=7.22 (d)	
H-1*	4.73 (2H) (s)	4.72 (2H) ^3J=4.52 (d)	4.78 (2H) (s)	4.75 (2H) (s)	4.83 (2H) (s)
H-3*-H-7*	7.23-7.36 (5H) (m)	7.28-7.36 (5H) (m)	7.35 (5H) (bs)	7.28-7.35 (5H) (m)	7.32-7.40 (5H) (m)
NH1 NH2	11.16 (1H) (bs)	11.22 (1H) (bs)	11.27 (1H) (bs)	11.27 (1H) (bs)	11.49 (1H) (bs) 8.11 (1H) (bs)
OH					

Table 4. ^{13}C NMR chemical shifts (δ/ppm) NSAID hydroxamic acids (HA) and their intermediates (IM)

	IBUPROFEN		FENOPROFEN		INDOMETHACIN		KETOPROFEN	BENZYLPROFEN	DICLOFENAC		
	HA	IM	HA	IM	HA	IM	IM	HA	HA	*N*1-Me-HA	IM
C-1	176.39	170.38	169.87	169.91	167.82	167.81	169.68	170.23	168.10	171.04	168.24
C-2	41.73	41.64	41.96	41.86	28.37	28.31	41.88	42.09	36.57	35.11	36.37
C-3	18.16	18.06	18.12	17.95	113.75	113.34	17.99	18.25	143.00	143.15	142.94
C-4	139.09	138.64	144.08	143.53	135.25	135.89	141.79	142.00	125.14	124.94	124.54
C-5	126.95	126.92	117.58	117.47	102.09	101.87	128.41	127.66	116.05	116.01	115.98
C-6	126.95	126.92	156.44 or 156.54	156.51	155.50	155.54	136.94 or 136.99	141.01	120.70	120.71	120.72
C-7	139.29	139.38	122.38	122.28	111.14	111.24	131.51	125.95	127.27	127.17	127.40
C-8	128.68	128.74	129.67	129.79	114.47	114.50	132.65	124.97	130.29	131.04	130.42
C-9	128.68	128.74	116.65	116.76	134.20	130.22	131.51	127.00		35.82	
C-10					134.20	134.17					
C-11					55.44	55.42					
C-12					13.32	13.27					
C-1'	44.22	44.19	156.44 or 156.54	156.51	166.33	166.53	195.64	41.18	137.17	137.19	137.06
C-2'	29.61	29.58			130.73	130.67	136.94 or 136.99	141.16			
C-2', C-6'			118.53	118.55					129.30	129.46	129.60
C-3', C-4'	22.13	22.11									
C-4'			123.39	123.40					124.94	125.09	125.19
C-4', C-6'							128.52				
C-3',C-5'			130.01	129.99					129.15	129.13	129.14
C-3', C-7'							129.56				
C-5'					137.58	137.57	128.55	128.23			
C-3',C-4', C-6',C-7'					129.02 131.13	129.01 131.12		128.40 128.65			
C-1*		76.49		76.58		76.76	76.59				76.84
C-2*		135.91		135.80		135.36	135.77				135.80
C-4*, C-6*											
C-3*, C-7*											
C-5*											
C-3*, C-7*		128.16 128.88		128.21 128.88		128.23 128.83 129.01	128.19 128.23 128.89				128.27 128.83

Table 5. The difference in ^{13}C NMR NMR chemical shifts for C=O group in NSAID, NSAID hydroxamic acids (HA) and intermediates (IM)

NSAIDs	Drug	Hydroxamic acid, HA	Intermediate, IM	Benzylprofen HA*	*N*1-Me-diclofenac HA**	ρ ppm NSAID/IM	ρ ppm NSAID/HA	ρ ppm IM/HA
Ibuprofen	175.37	179.39	170.38			4.99	-4.02	-9.01
Fenoprofen	175.80	169.87	169.91			5.89	5.93	0.04
Indomethacin	171.97	167.82	167.81			4.16	4.15	-0.01
Ketoprofen	175.06		169.88	170.23*		5.18	4.83*	-0.35*
Diclofenac	173.29	168.10	168.24		171.04	5.05	5.19 (2.25**)	0.014 (-2.8**)

* Benzylprofen was isolated from the reaction of ketoprofen IM with H_2/Pd, instead of expected ketoprofen HA. ** HA of N1-Me-diclofenac derivative.

Table 6. NOESY interactions in DMSO-d_6 solution of NSAID hydroxamic acids (HA) and their intermediates (IM)

	Observed proton	NOESY interactions (Relative intensity)
Ibuprofen HA	H-2 H-1'	NH (s); OH (s); H-5,6 (s); H-3 (s) H-8,9 (s); H-2' (s); H-3',4' (s)
Ibuprofen IM	H-2 H-3 H-3',4' H-1*	H-5,6 (s) H-5,6 (s) H-1' (s); H-2' (s); H-8,9 (s); H-5,6 (w) H-2 (s); H-3'-7' (s); NH (s)
Fenoprofen HA	H-2 H-3	H-3 (s); OH (s); NH (w); H-2 (s); H-5 (s); H-2',6' (s); OH (w); NH (w)
Fenoprofen IM	H-2 H-3 NH	H-5 (s); H-2',6' (s); NH (s) H-2 (s); H-5 (s); H-2',6' (s) H-2 (s); H-3 (w); H-5 (w); H-1'' (s); H-3''-7'' (s)
Indomethacin HA	H-2 NH	NH (s); OH (s) OH (s)
Indomethacin IM	H-2 H-8 H-12 H-1'	H-5 (s); H-12 (s); NH (s) H-3'' i H-7'' (s); H-4'' i H-8'' (s) H-2 (s); H-3'' i H-7'' (s); H-4'' i H-8'' (s); NH (s) H-3''-7'' (s); NH (s)
Ketoprofen IM	H-2 H-1*	H-3 (s); H-5 (s); H-7 i H-9 (s); H-8 (s); NH (s) H-3''-7'' (s); NH (s)
Benzyprofen HA	H-2 OH	H-1' (s); NH (s); OH (w) H-2 (s); NH (s); H-1' (w)
Diclofenac HA	H-2	NH_1 (s); OH (s)
*N*1-Me-diclofenac HA	H-9	H-2 (s); H-8 (s); NH (w); OH (w)
Diclofenac IM	H-2 H-5 H-1*	H-8 (s); NH (s); OH (s); H-1'' (s); H-7' (w) H-2 (s); H-7' (s) H-2 (s); H-3''-7'' (s); NH (s); OH (s)

Table 7. NOESY and HMBC interactions in NMR spectra of ibuprofen IM and HA, and fenoprofen IM and HA, in part of their profane moieties, R-CH(CH_3)CONH-R'.

	H-atom	NOESY interaction		HMBC interaction	
		yes	no	yes	no
Ibuprofen HA	OH CH_3	H-2 H-8 and H-9	CH_3 H-5 and H-6	C=O and OH (s)	C=O and NH
Ibuprofen IM	CH_3	H-8 and H-9	H-5, H-6 and NH		C=O and NH
Fenoprofen HA	OH NH	H-2 (s), CH_3 (w) H-2 (s), CH_3 (w), H-5,7,8,9 (vw)		C=O and OH (vs)	C=O and NH
Fenoprofen IM	NH CH_3	H-2 (s), CH_3 (w), H-5,7,8,9 (vw), H-5 (s)		C=O and NH (s)	

s - strong, vs - very strong , w - weak, vw - very weak

^1H and ^{13}C NMR spectral data (Table 1 to 3) have confirmed proposed chemical structures of investigated compounds.

In FTIR spectra of synthesized compounds the H-bond was observed in the range of -OH and -NH stretching vibrations and in the range of C(1)=O stretcing vibrations of hydroxamic acids, showing te shift of these vibrations to less vave numbers, ν (cm^{-1}) comparing to C(1)=O in NSAID and intermediates (IM). (Table 1, Fig. 1, Fig. 2)

^1H NMR spectra revealed that N(1)H protons in all intermediates (IM) are from the 11.16 ppm to 11.49 ppm, while the same protons in hydroxamic acids appear as a broad signals in the range from 7.84 ppm to 8.84 ppm, and hydroxyl protons of hydroxamic moiety from 9.02 ppm to 10.66 ppm. These protons are shifted up in the field for cca 1.5 ppm in comparison to corresponding carboxyl protons.

^{13}C NMR spectra of investigated compounds revealed that carbonyl groups, C(1)=O, in all NSAIDs were in the range from 171.97 ppm to 175.80 ppm, while in their hydroxamic acid's intermediates (IM) from 167.81 ppm to 170.38 ppm and in hydroxamic acids (HA) from 167.82 ppm to 179.38 ppm.

The most significant differences in chemical shifts of C(1)=O were founded in order ibuprofen, ibuprofen IM and ibuprofen HA.

Contrary to all other NSAIDs, ibuprofen HA showed significant down field shift as compared to ibuprofen (NSAID/HA = -4.02 ppm) and to ibuprofen hydroxamic acid intermediate (IM) (IM/HA = - 9.01 ppm).

The C(1)=O chemical shifts of all NSAID hydroxamic acids (except of ibuprofen HA) and their intermediates (IM) were shifted up in the field, amounting to 4.16-5.89 ppm, in comparison to drug itself. In these compounds -NH-OBn and -NHOH groups shield their neighbouring carbonyl groups, with results of C(1)=O chemical shifts in upper field, i.e. at less number. No significant differences between chemical shifts for C(1)=O were found in intermediates (IM) and their hydroxamic acid (HA) of fenoprofen, indomethacin, ketoprofen and diclofenac.

It is known that ibuprofen forms an intermolecular H-bonds which is important to its pharmacological effect in COX inhibition.

NMR spectra in this study showed that ibuprofen hydroxamic acid exist in DMSO-d_6 solution in the form with intra-molecular H-bond. The formation of 5-member-ring in ibuprofen HA due to H-bond between hydrogen proton of hydroxyl group and carbonyl oxygen results in formation of 5-member-ring. Due to the fact that H-bonding weakens the electron density of carbonyl group, C(1)=O is shifted down field in ^{13}C NMR, in comparison to chemical shift of C(1)=O in ibuprofen IM and ibuprofen itself. This fenomen of ibuprofen HA, in comparison to other investigated hydroxamic acids, could be explained by inductive effect of *i*-butyl substituent in p-position toward to 2-propyl carboylic acid moiety and its derivatives.

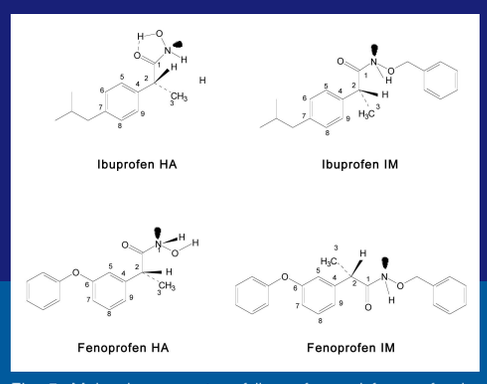

Fig. 5. Molecular geometry of ibuprofen and fenoprofen hydroxamic acids and their intermediates derived on the basis of NOESY and HMBC interactions.

Fig. 1. A parallel display of FTIR spectra fenoprofen HA (black line) and fenoprofen IM (red line)

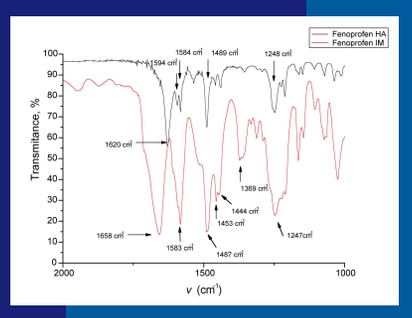

Fig 2. A parallel display of part FTIR spectra of fenoprofen HA (black line) and fenoprofen IM (red line), the region from 2000 to 1000 cm^{-1}, showing shift of C(1)=O stretching vibrations towards less ν (cm^{-1}) for 30 cm^{-1}

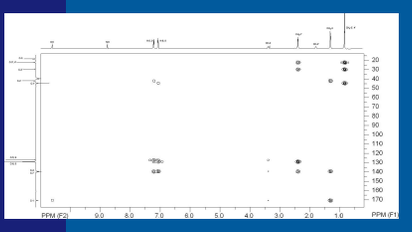

Fig. 3. HMBC spectrum of ibuprofen hydroxamic acid (HA)

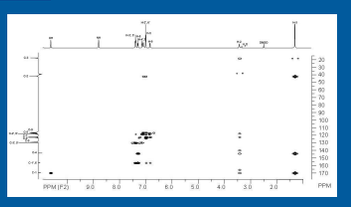

Fig. 4. HMBC spectrum of fenoprofen hydroxamic acid (HA)

CONCLUSIONS

- The structures of synthesized NSAID hydroxamic acids and their intermediates were determined by means of FTIR (Table 1) , 1D- and 2D-dimensional, homo- and heteronuclear ^1H and ^{13}C NMR spectroscopy (Table 2 to Table 7).
- The H-bonding was observed in solid state in FTIR spectra and in DMSO-d_6 solution in NMR spectra.
- Although there is possibility of of keto-iminol tautomerism in the hydroxamic acid moiety, all investigated compounds recorded in DMSO-d_6 showed keto form of tautomer (Table 1 and Table 3).
- On the basis of NOESY NMR spectral data it was concluded that the hydroxyl proton of ibuprofen hydroxamic acid is intra-molecular H-bonding with C(1)=O, thus forming 5-membered ring, while in other NSAID HA these protons are more likely undergo to inter-molecular H-bonding due to inductive effect of substituents and their reflection on molecular geometry of their profane moiety which is not favourable for intra-molecular H-bonding (Fig. 3).
- HMBC spectra of all investigated HAs showed interactions of hydroxamic hydroxyl proton with C1 through three bonds, while interaction NH proton with the same C1 atom was not observed, except in HMBC spectra of fenoprofen IM. All other HMBC spectra of IMs does not show interactions of NH proton with C1, through two bonds, what maybe indicate an involvement of this proton in other interactions, i.e inter-molecular H-bonding (Fig. 3 and Fig. 4)
- The molecular geometry of investigated hydroxamic acids and their intermediates in DMSO-d_6 solution, results from different inductive effect of substituent either in *p*- or *m*-position towards profane moiety, as well as the possibility of forming H-bond (Fig. 5).
- The results of this study of structural behaviour of NSAIDs, hydroxamic acids (HA) and their intermediates (IM) in solid state and in solution could be useful and also contribute to investigation of NSAID specific inhibition toward either COX-1 or COX-2 enzymes.

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