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Research Article

DETERMINATION OF BIOLOGICAL ACTIVITIES AND ACIDITY AT DRUG ACTIVE SUBSTANCE IN SOME BENZIMIDAZOLE DERIVATIVES

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ABSTRACT

Some benzimidazole derivatives as known drug active substance were evaluated for their biological activities such as antibacterial, antiviral and antifungal activities. In addition, acid dissociation constants (pKa) were determined experimentially with potentiometric titration method and theoretically with SPARC computer programme about state acidity for these six compounds.

Keywords: Antibacterial, antiviral , antifungal, acidity, drug active substance.

INTRODUCTION

Benzimidazoles are an important group of heterocyclic compounds in the field of medicinal chemistry because they frequently have interesting biological and pharmacological properties. For example, in 1944, Wayne Walley, one of the leading exponents of antimetabolite therapy, noted the resemblance of benzimidazole to adenine and speculated that it might act as an adenine antimetabolite.[1] Benzimidazole is a fused heterocycle of benzene with imidazole that possesses a larger conjugated system and electron-rich properties higher than those of imidazole,[2] thiazole,[3] oxazole,[4] triazole,[5-7] and tetrazole.[8]

These special structures make benzimidazole-based derivatives easily interact with various active targets and exhibit a broad range of properties in medicinal chemistry, including antihelmintic, antihistaminic, anticancer, antiviral, antiinflammatory, antiproliferative, antioxidant, and anticoagulant bioactivities.[9-12] Recently, many benzimidazole derivatives have been successfully developed in clinics, such as the antihistaminic Astemizole, anti-anabrotic Omeprazole, antihypertensive Candesartan, and antiparasitic Albendazole. Many studies have demonstrated that benzimidazoles as analogues of purines can compete with purines to efficiently prevent the synthesis of nucleic acids and proteins, thereby inhibiting the growth of various microorganisms and killing them. This suggests that, according to their different mechanisms, benzimidazoles should possess great potential as a new type of antimicrobial agent. This possibility has encouraged many studies to explore novel benzimidazole derivatives to new molecular scaffolds with high efficiency, broad spectra, and low toxicity.[9] Recently, several active benzimidazoles have been developed that display good or even superior bioactivity.[13-15]

Acid dissociation constants are very important parameters, which can provide critical information about chemical properties such as acidity.[16-18] Hence, the relationship between the acid dissociation constants and structure in molecules is important.[17-19] Acid dissociation constants are also important parameters for the selection of the optimum conditions in the development of analytical methods[20,21] and provide information about the stereo chemical and conformational structures of active centers of enzymes.[22] Acid dissociation constants are determined by several methods such as potentiometric[20] spectroscopic,[19] electrophoretic methods[19,23] and theoretical.[22] Thus, the acid dissociation constants of these compounds is still of great interest. Benzimidazole derivatives (**1a-f**, **2a-f**, **3a-f**) in this studied were prepared according to the literature.[1]

EXPERIMENTAL SECTION

Pharmacology

Standard Strains; Antibacterial and antifungal activities of 18 compounds **1a-f**, **2a-f**, **3a-f** (Figure 1) were tested against 3 bacterial strains

Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923 and Bacillus subtilis ATCC 6633, and 3 fungal Candida krusei ATCC 6258, Candida parapsilosis ATCC.

Comp.	R_1	R_2	R_3
a	-Cl	-H	-H
b	-H	-Cl	-H
c	-H	-H	-Cl
d	$-CH_3$	-H	-H
e	-H	$-CH_3$	-H
f	-H	-H	$-CH_3$

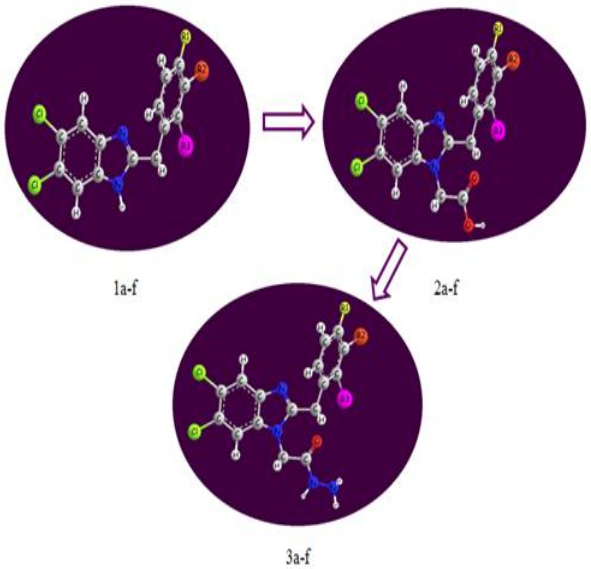


Fig. 1: Synthetic route of compounds 1a-f, 2a-f, and 3a-f [1]

Antibacterial Activity Testing

Agar well diffusion assay and broth microdilution method were used to test of antibacterial activity of compounds[24,25]. Antifungal activity of compounds against fungal standard strains was evaluated only with broth microdilution method. Each compound was tested twice.

Agar Well Diffusion Assay

Agar well diffusion assay was performed as described.[24] Briefly, the chemical compounds were weighed and dissolved in dimethyl sulphoxide (DMSO) to prepare the solution of 4 mg/mL. Ampicillin (100 µg/mL) and DMSO solutions were used as controls. Ten µL of each bacterial suspensions spread with a sterile glass L-rod spreader onto surface of Mueller-Hinton agar plates. Then, six mm in diameter wells were cut off from the agar by using sterile glass-made pipettes attached to a vacuum pump, and 0.1 mL of each chemical and controls were delivered into the wells, constituting 0.4 mg compound per well, 10 µg for ampicillin. The next day, plates were examined for any zones of growth inhibition.

Broth Microdilution Method

Minimal inhibitory concentration (MIC) values determined by broth microdilution method described as elsewhere.[24] Briefly, 100 µL of broth media (Mueller-Hinton Broth (MHB) for bacteria, YEPD for fungi) was delivered into microwell plate. To the first wells of rows of the plates, 100 µL of compound suspension and controls were added and serially diluted. A hundred µL of bacterial suspensions was placed into each well, giving final concentration of chemicals 1, 0.5, 0.25, 0.125, 0.062, 0.031, 0.016, 0.008, 0.004, 0.002, 0.001, and 0.0005 mg/mL. Ampicillin (100 µg/mL) and DMSO were used as controls. After the incubation at 37ºC for 16-18 hours, MICs were determined as the lowest concentration of compound and controls at which no visible growth was observed.

Antifungal Activity Testing

Antifungal susceptibility testing was performed using broth dilution method according to EUCAST E.Dis.7.1.[26] Assay was performed as above but final concentration of chemicals started with 100 and finished with 0.04 µg/mL. Fluconazole (100 µg/mL) and DMSO were used as controls.

Acidity

Potentiometric Titration

In this study, Orion Model 720A pH ion meter, fitted witha combined pH electrode (Ingold) was used for potentiometric titrations. An Ingold pH electrode was preferred because of the advantage. A magnetic stirrer, a semi-micro burette and a 25 mL beaker were also used in titrations. All the chemicals were supplied from Merck. Before potentiometric titrations (Figure 2), the pH meter was calibrated according to the instructions supplied by the manufactures of the pH meter. In this section, the pH electrode calibrated with 4, 7, 10 and 12 pH tampon solution.

Fig. 2: System of potentiometric titration cell used in studied

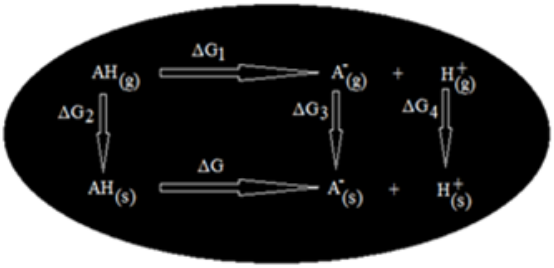
In this study, six benzimidazole derivatives (5,6-dichloro-2-(2-chloro benzyl)-1*H*-benzimidazole (**1a**), 5,6-dichloro-2-(3-chlorobenzyl)-1*H*-benzimidazole (**1b**), 5,6-dichloro-2-(4-chlorobenzyl)-1*H*-benzimidazole (**1c**), 5,6-dichloro-2-(2-methylbenzyl)-1*H*-benzimidazole (**1d**), 5,6-dichloro-2-(3-methylbenzyl)-1*H*-benzimidazole (**1e**), 5,6-dichloro-2-(4-methylbenzyl)-1*H*-benzimidazole (**1f**) were synthesized in Recep Tayyip Erdoğan University Organic Chemistry Research Laboratory and published.[1]

For each compound that would be titrated, the 0.001 M solution was separately prepared in each non-aqueous solvent (isopropyl alcohol, *N,N*-dimethylformamide, *tert*-butyl alcohol and acetonitrile). During the titrations, the titrant was added in increments of 0.05 mL after each stable reading, and mV values were recorded. After purifications, isopropyl alcohol was used to prepare 0.05 N tetrabutylammonium hydroxide (TBAH). For all potentiometric titrations, 0.05 N TBAH in isopropyl alcohol, which was prepared from 0.1 N TBAH by dilution, was used. The mV values, that were obtained in pH meter, were recorded. Graphs were drawn by obtained from all data and end point is determined by $\Delta E/\Delta V$ - (TBAH, ml), $\Delta^2E/\Delta V^2$ - (TBAH, ml) and $\Delta V/\Delta E$ - (TBAH, ml) graphics. Finally, the half-neutralization potential (HNP) values were by drawing these The ionization of weak acid (HA) is given for the gas and solvent phase in Figure 3. Calculation of pKa were made using the free energy changes in the thermodynamic cycle. Respectively ΔG_1, ΔG_2, ΔG_3 and ΔG_4 are calculated for find the ΔG (in solvent phase). Then, pKa is calculated using the equation with calculated ΔG. In this paper, we describe the details of the SPARC reactivity computational methods and its performance on predicting the pKa values of these benzimidazole derivatives in comparison with experimental values.

graphic and pKa values were determined according to half-neutralization method.

SPARC Computer Program

The computer program SPARC (SPARC Performs Automated Reasoning in Chemistry) was developed to predict numerous physical properties such as vapor pressure, distribution coefficient, and GC retention time as well as chemical reactivity parameters such as pKa and electronaffinity. SPARC predicts both macroscopic and microscopic pKa values strictly from molecular structure using relatively simple reactivity models.[21] SPARC computer program is based on the thermodynamic cycle (Figure 3) as shown below.



$\Delta G = [((\Delta G_3 + \Delta G_4) - \Delta G_2) + \Delta G_1]$
$\Delta G = -2.303 \cdot R \cdot T \cdot \log Ka$
Fig. 3: Thermodynamic cycle

RESULTS AND DISCUSSION

Pharmacology

Antibacterial Activity Results

Among the compounds tested only **1e** and **3f** showed antibacterial activity (Table 1). No inhibitory effect was detected for other compounds less than 4 mg/mL concentration.

Antifungal Activity Results

The results of antifungal activity of the compounds are shown in Table 2 and indicates that only the compound **3f** showed activity against *C. parapsilosis* ATCC 22019 (a sensitive yeast) with a 50 µg/mL MIC value (Table 2). No inhibitor activity was seen against *C. krusei* ATCC 6258 (a resistant yeast) and A. *niger* DSMZ 1988 (a mold) by broth microdilution method.

Table 1: Antibacterial activity of chemical compounds

Comp.	*E.coli*		*S.aureus*		*B.subtilis*	
	ZD[a]	MIC[b]	ZD	MIC	ZD	MIC
1a	-[c]	>1	-	>1	-	>1
1b	-	>1	-	>1	-	>1
1c	-	>1	-	>1	-	>1
1d	-	>1	-	>1	-	>1
1e	-	>1	12	0.25	14	0.125
1f	-	>1	-	>1	-	>1
2a	-	>1	-	>1	-	>1
2b	-	>1	-	>1	-	>1
2c	-	>1	-	>1	-	>1
2d	-	>1	-	>1	-	>1
2e	-	>1	-	>1	-	>1
2f	-	>1	-	>1	-	>1
3a	-	>1	-	>1	-	>1
3b	-	>1	-	>1	-	>1
3c	-	>1	-	>1	-	>1
3d	-	>1	-	>1	-	>1
3e	-	>1	-	>1	-	>1
3f	-	>1	10	0.25	10	0.25
Ampisilin	18	1.562 µg/mL	30	0.781 µg/mL	25	1.562 µg/mL
DMSO/PBS (1/10)	-	-	-	-		-

[a]Zone diameter (mm),
[b]Minimal Inhibitory Concentration (mg/mL), [c]No inhibition

Table 2: Antifungal activity of chemical compounds

Compound Code	*C.parapsilosis* MIC[a]	*C.krusei* MIC	*A.niger* MIC
1a	≥100	≥100	≥100
1b	≥100	≥100	≥100
1c	≥100	≥100	≥100
1d	≥100	≥100	≥100
1e	≥100	≥100	≥100
1f	≥100	≥100	≥100
2a	≥100	≥100	≥100
2b	≥100	≥100	≥100
2c	≥100	≥100	≥100
2d	≥100	≥100	≥100
2e	≥100	≥100	≥100
2f	≥100	≥100	≥100
3a	≥100	≥100	≥100
3b	≥100	≥100	≥100
3c	≥100	≥100	≥100
3d	≥100	≥100	≥100
3e	≥100	≥100	≥100
3f	50	≥100	≥100
Fluconazole	0.1	50	50
DMSO	-	-	-

[a]Minimal Inhibitory Concentration (µg/mL)

Acidity

Potentiometric Titration

In this study, all compounds were titrated potentiometrically with TBAH in isopropyl alcohol, *N,N*-dimethylformamide, *tert*-butyl alcohol and acetonitrile. The mV values read in each titration were drawn against TBAH volumes (mL) added and potentiometric titration curves were formed for all the cases. Experiments were repeated 3 times in each experiment. Standard deviations was calculated for this three experiments. Calculations were performed within 95 % confidence interval. From the titration curves (Figure 4), the HNP (half-neutralization potential) values were measured and the corresponding pKa values were calculated. The HNP values and the corresponding pKa values of all triazole derivatives, obtained from the potentiometric titrations with 0.05 M TBAH in isopropyl

alcohol, *N,N*-dimethyl formamide, *tert*-butyl alcohol and acetonitrile and pKa for all compounds were calculated theoretically with SPARC computer programme. All pKa and HNP values are presented in Table 3. Theoretical and experimental pKa values were comparisoned as an example of the compound **1a** in Figure 5.

When the dielectric permittivity of solvents is taken into consideration, the acidic arrangement can be expected as follows: *N,N*-dimethylformamide (ε = 36.7) > acetonitrile (ε = 36.0) > isopropyl alcohol (ε = 19.4) > *tert*-butyl alcohol (ε = 12.0). But, in this studied that it is observed isopropyl alcohol > *tert*-butyl alcohol > acetonitrile > *N,N*-dimethylformamide for compound **1b**, **1c** and **1e**, isopropyl alcohol > *tert*-butyl alcohol > *N,N*-dimethylformamide > acetonitrile for compound **1a**, acetonitrile > isopropyl alcohol > *tert*-butyl alcohol > *N,N*-dimethylformamide for compound **1f**, isopropyl alcohol > acetonitrile > *tert*-butyl alcohol > *N,N*-dimethylformamide for compound **1d**.

When dielectric constant is examined according to the acidity forces (amphiprotic solvents the dielectric constant of isopropyl alcohol and *tert*-butanol, respectively, 19.4 and 12.0). The acidity of the compounds are expected more acidic for high dielectric constant has solvent (isopropyl alcohol). In this study, it is obtained a result of compounds **1a**, **1b**, **1c**, **1d**, **1e** and **1f** data were found to be suitable in this order. When dipolar aprotic solvents is considered, the increase in strength of the acidity is expected as *N,N*-dimethyl formamide > acetonitrile. Compound **1a** was observed to follow this order.

When analyzed according to autoprotolysis constant, weak acidic property is showed in isopropyl alcohol (pKs: 20.6), *N,N*-dimethylformamide (pKs: 18.0) and *tert*-butanol (pKs: 22.0) but strong acidic property (except compound 9) is showed in acetonitrile (pKs: 33.0) for all compounds. By the time analyzed according to the functional group (-R) effect, it has showed very small effect for acidic protons due to the distance. By the time compounds were analyzed by each solvent, acidity strength decrease; **1b** > **1c** > **1e** > **1a** > **1d** > **1f** in isopropyl alcohol, **1c** > **1a** > **1b** > **1e** > **1d** > **1f** in *N,N*-dimethylformamide, **1e** > **1c** > **1b** > **1d** > **1a** > **1f** in *tert*-butyl alcohol and **1f** > **1d** > **1e** > **1a** > **1c** > **1b** > in acetonitrile as observed. Differentiated all compounds showed in the studied solvents when investigated the effect of the leveling and differentiated. When compared theoretical results with potentiometric results was obtained by the half-neutralization method, errors were found to be between 0.51% (compound **1a** in *tert*-butily alcohol) and -18.52% (compound **1c** in *N,N*-dimethylformamide). Percentage error values of between the theoretical values and the experimental values are given in Table 3.

Table 3: Half-Neutralization Potential (HNP) Values, experimental and theoretical the corresponding pKa values of all studied molecules

Comp.	Solvent	pKa (Experiential)	HNP (mV)	pKa (Theoretical)	Relative Error, %
1a	Isopropyl alcohol	13.03 ± 0.04	-357.4 ± 0.8	12.08	7,29
	N,N-Dimetilformamid	14.03 ± 0.06	-415.8 ± 2.9	16.35	-16,54
	Tert-Butily alcohol	13.63 ± 0.03	-394.8 ± 5.5	13.70	-0,51
	Acetonitrile	14.44 ± 0.02	-439.9 ± 1.3	15.29	-5,89
1b	Isopropyl alcohol	12.44 ± 0.06	-318.8 ± 1.3	12.10	2,73
	N,N-Dimetilformamid	14.05 ± 0.04	-416.3 ± 2.3	16.37	-16,51
	Tert-Butamol	13.37 ± 0.07	-378.2 ± 4.5	13.72	-2,62
	Asetonitril	14.04 ± 0.09	-416.5 ± 6.1	15.32	-9,12
1c	Isopropyl alcohol	12.91 ± 0.08	-348.5 ± 3.6	12.10	6,27
	N,N-Dimetilformamid	13.82 ± 0.04	-403.6 ± 2.3	16.38	-18,52
	Tert-Butily alcohol	13.32 ± 0.06	-373.7 ± 4.9	13.73	-3,08
	Acetonitrile	13.65 ± 0.07	-397.0 ± 5.8	15.32	-12,23
1d	Isopropyl alcohol	13.20 ± 0.09	362.7 ± 8.1	12.30	6,82
	N,N-Dimetilformamid	14.28 ± 0.05	430.6 ± 4.9	16.56	-15,97
	Tert-Butily alcohol	13.51 ± 0.08	383.5 ± 7.4	13.91	-2,96
	Acetonitrile	13.30 ± 0.06	371.8 ± 5.1	15.52	-16,69
1e	Isopropyl alcohol	12.92 ± 0.05	-347.3 ± 6.5	12.28	4,95
	N,N-Dimetilformamid	14.09 ± 0.08	-419.4 ± 5.8	16.55	-17,46
	Tert-Butily alcohol	12.98 ± 0.10	-354.2 ± 7.6	13.90	-7,09
	Acetonitrile	13.39 ± 0.02	-378.0 ± 0.8	15.50	-15,76
1f	Isopropyl alcohol	13.57 ± 0.06	-388.1 ± 5.2	12.29	9,43
	N,N-Dimetilformamid	14.54 ± 0.04	-445.4 ± 7.3	16.56	-13,89
	Tert-Butily alcohol	14.14 ± 0.07	-422.8 ± 4.9	13.92	1,56
	Acetonitrile	13.28 ± 0.05	-371.6 ± 6.8	15.51	-16,79

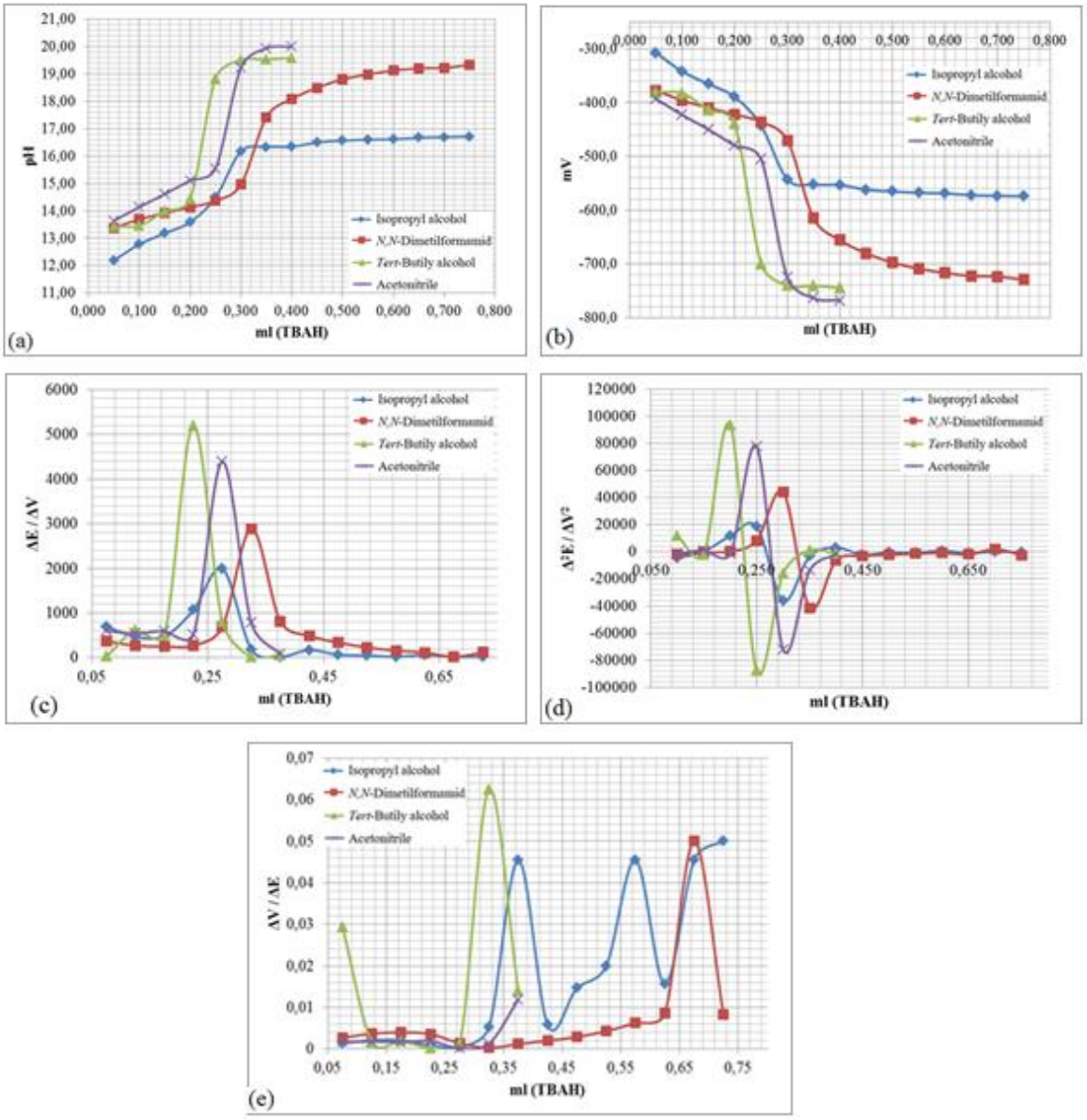


Fig. 4: (a) pH-ml (TBAH), (b) mV-ml (TBAH), (c) ΔE/ΔV-ml (TBAH), (d) $\Delta^2E/\Delta V^2$-ml (TBAH) and (e) ΔV/ΔE-ml (TBAH) potentiometric titration curves of 0.001 M solutions of compound 1a titrated with 0.05 M TBAH in isopropyl alcohol, *N,N*-dimethylformamide, *tert*-butyl alcohol and acetonitrile at 25 °C.

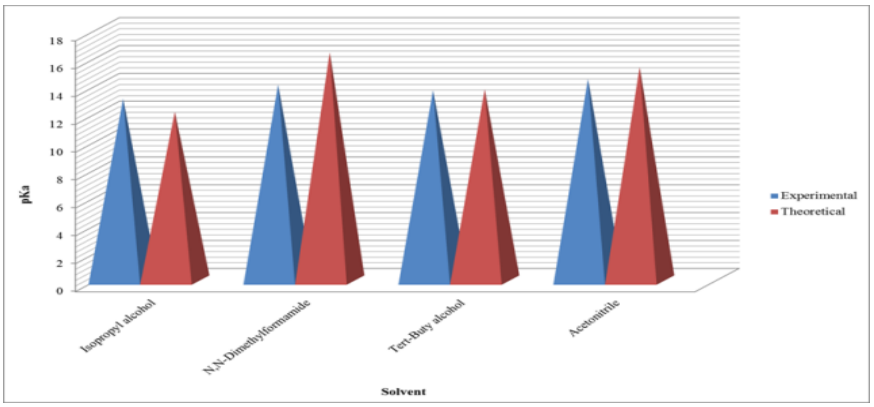


Fig. 5: Experimental and theoretical results compared for compound 1a

CONCLUSIONS

The acidity of a compound depends on mainly two factors, i.e. solvent effect and molecular structure. Half-neutralization potential (HNP) values and corresponding pKa values obtained from the potentiometric titrations rely on the non-aqueous solvents and theoretically with SPARC computer programme. Different benzimidazole derivatives will evaluated for biological activities except antibacterial, antiviral and antifungal activities.

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