Facile Syntheses of Novel Salts of a Triazole Antifungal Agent with Enhanced Solubility

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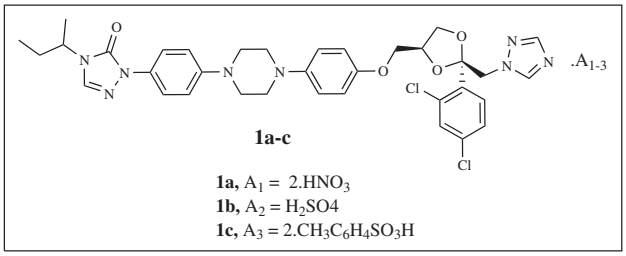

Acid addition salts of a triazole antifungal agent itraconazole were prepared with an aim to enhance the aqueous solubility and dissolution characteristics of the drug. Nitric, sulfuric, and *p*-toluenesulfonic acid addition salts (**1a–c**) were prepared using facile synthetic procedures. The solubility of the salts in water was found to be 24, 22, and 58 mg/mL, respectively, for **1a**, **1b**, and **1c**, which is significantly higher than that of itraconazole (1 ng/mL). In addition to this, solubility of the salts in simulated gastric fluid (SGF) and other pharmaceutically used solvents such as ethanol and propylene glycol was also improved to a great extent. Also, it was observed that from **1a**, **1b**, and **1c**, approximately 33, 26, and 45% drug was released in SGF in 3 h, whereas less than 10% drug was released from the free base form.

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INTRODUCTION

Triazole antifungals are the third generation of antifungal drugs and are known to possess a wide spectrum of antifungal activity against a variety of pathogenic fungi (Fig. 1). Although very potent, some of the triazole drugs are insoluble in water and most other pharmaceutically acceptable solvents, thus resulting in their poor bioavailability and affecting their usability in many drug delivery systems [1]. Several efforts to improve solubility have been reported in recent past, most important of which involve use of solid dispersions with polymers, microemulsion systems, and complex formation with crown ethers to enhance apparent aqueous solubility [2–4]. These techniques vastly improve the solubility profile, but they add to the cost of the final product and involve several tedious experimental stages and unit operations. Administration of these drugs as acid addition salts could be a logical approach to improve the solubility and hence bioavailability of these drugs.

We chose itraconazole as a model insoluble drug of this class and investigated the prospect of salification and associated physicochemical improvement. Itraconazole contains a piperazine moiety with p*K*a 3.7, while its other ionizable nitrogen atoms are not protonated at extremely acidic pH environments [5,6]. Although formation and solubility enhancement of itraconazole by forming hydrochloride salt has been recently reported [7], this salt may not work in *in vivo* conditions mainly because of common ion effect of chloride ions as described by many workers [8,9]. Nitric, sulfuric, and *p*-toluenesulfonic acid were used to prepare the acid addition salts of itraconazole. The salts were prepared using facile synthetic procedures and were characterized in terms of solubility and dissolution rate enhancement.

RESULTS AND DISCUSSIONS

Nitric, sulfuric, and *p*-toluenesulfonic acids were used for carrying out the synthesis of acid addition salts of itraconazole (Scheme 1). Methanolic solutions (50% w/w) of 2 equimolar amount of monoprotic nitric and *p*-toluenesulfonic acid and 1 equimolar amount of diprotic sulfuric acid in the presence of itraconazole solution in chloroform under reflux conditions yielded corresponding itraconazolium salts **1a–c** in very good yields. The downfield shift of especially the eight piperazine ring protons for itraconazolium salts was observed as compared with itraconazole. This could be attributed to the deshielding of piperazine protons caused by the salt formation at nitrogen atoms of piperazine. Itraconazole free base exhibited two doublets for the eight piperazine protons at δ 3.15–3.17 and δ 3.22–3.24, whereas the two doublets were encountered for **1a** at δ 3.23–3.25 and δ 3.36–3.38 and for **1b** at δ 3.22–3.25 and δ 3.35–3.38. In

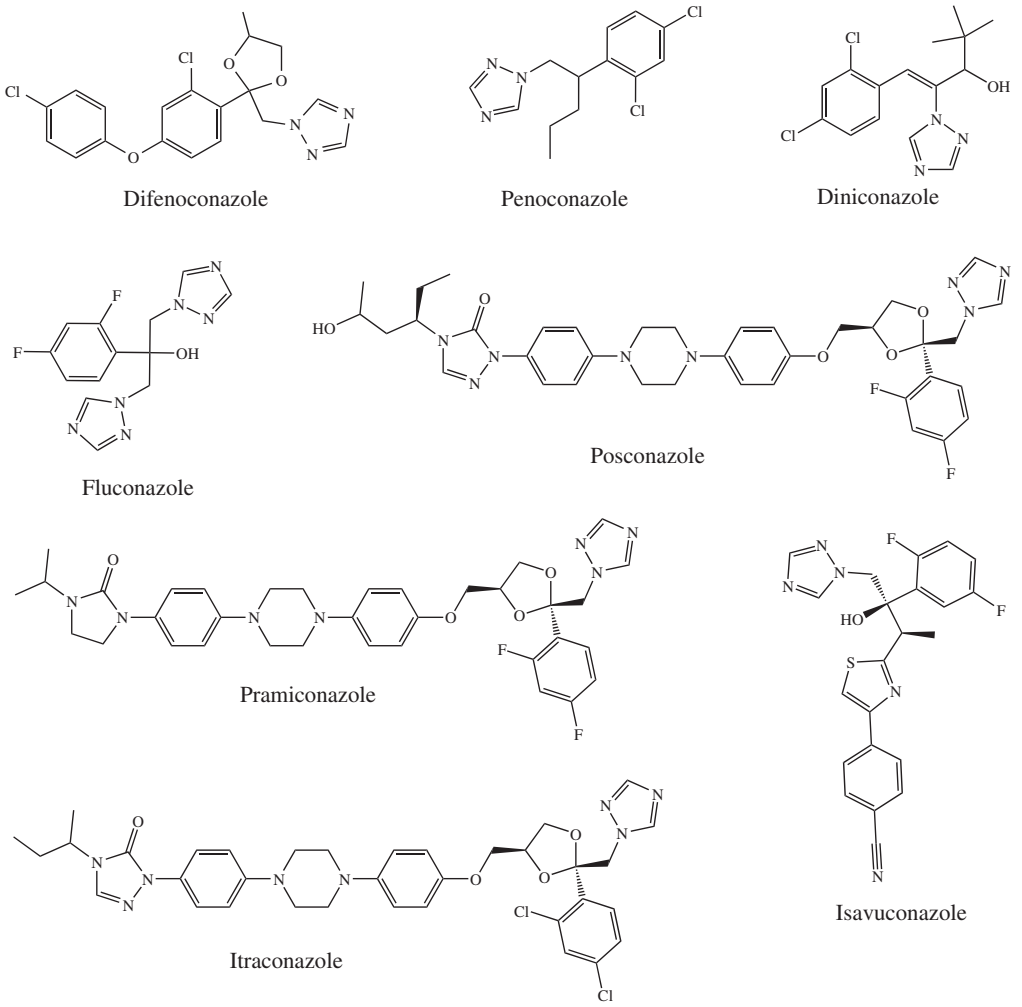
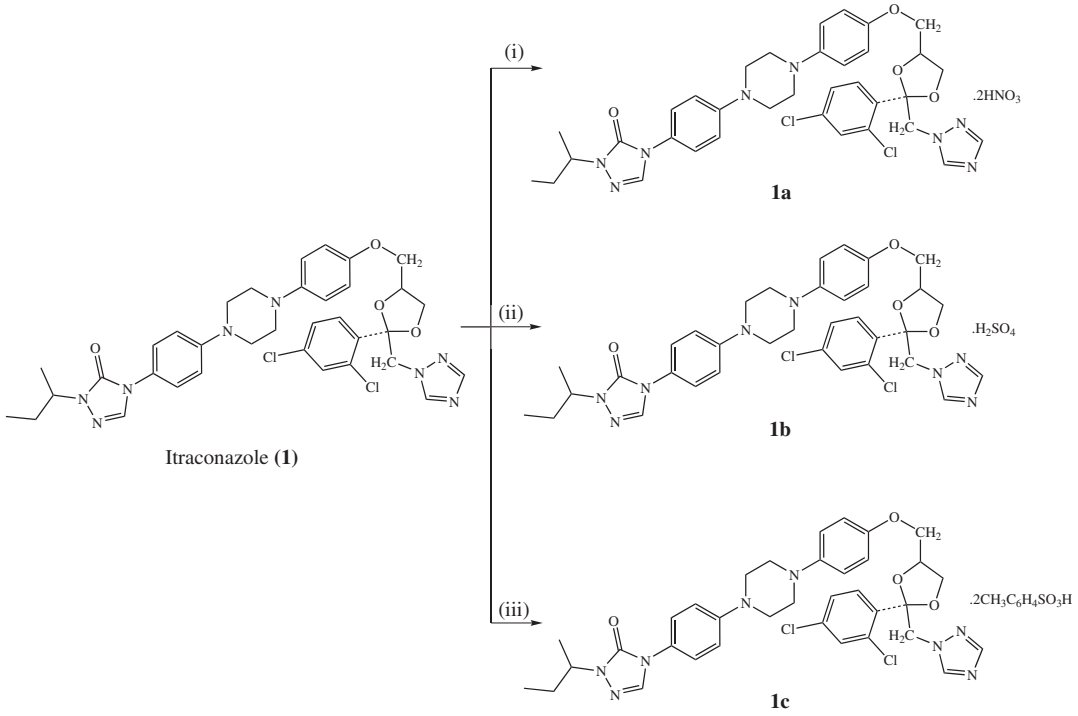


Figure 1. Triazole antifungal agents.

case of **1c**, the peaks for eight protons were found to be merged along with other protons in the form of a multiplet occurring at 3.90–3.93. In addition to this, **1c** also showed other peaks attributable to itraconazole ditoluenesulfonate structure, mainly a singlet at δ 2.35 due to the presence of two toluene methyl groups. The melting points of itraconazolium salts were determined to be notably different from itraconazole. The powder X-ray diffractogram of itraconazole exhibited characteristic sharp peaks [Fig. 2 (**1**)]. This pattern was found to be in concordance to earlier reports present in open literature [10,11]. The X-ray diffraction (XRD) data of salts when compared with that of itraconazole exhibited entirely new diffraction patterns with partial (**1a** and **1c**) and complete (**1b**) loss of crystallinity of itraconazole after salt formation [Fig. 2 (**1a–c**)]. The optical micrographs exhibited entirely new topography of the **1a–c**. The characteristic acicular or needle shaped particles were observed for itraconazole [Fig. 3 (**1**)]. Similar morphology of itraconazole has been mentioned in some previous reports [4,7]. Itraconazolium salts **1a–c** exhibited smaller particles having different characteristics. Salt **1a** appeared to be having appreciably equant shape, whereas **1b** and **1c** possessed plated and lathed particle morphologies [Fig. 3 (**1a–c**)]. Irrespective of the observed particle shapes, the reduction in particle size could be considered to be a merit as it exposes a large surface area to surrounding liquids and, therefore, leads to increased solubility and dissolution rate.

The solubility studies revealed remarkable increase in solubility of salts **1a–c** (Table 1). The aqueous solubility of itraconazole is reported to be 1 ng/mL at neutral pH. The aqueous solubility for itraconazole salts was enhanced to about 58,000 times for the **1c**. Similarly, about 24,000 times enhancement in aqueous solubility for **1a** and 23,000 times for **1c** was observed. Also in comparison to itraconazole free base, about 300-fold,

Scheme 1. Synthesis of **1a–c**. (i) HNO_3 : CH_3OH (1:1), $CHCl_3$, 10 min, reflux; (ii) H_2SO_4 : CH_3OH (1:1), $CHCl_3$, 15 min, reflux; (iii) $CH_3C_6H_4SO_3H$: CH_3OH (1:1), $CHCl_3$, 25 min, reflux.



275-fold, and 800-fold increase of solubility in ethanol and 8, 7, and 31 times increase of solubility in propylene glycol was observed for **1a**, **1b**, and **1c** respectively.

The *in vitro* release profiles for itraconazolium salts demonstrated faster release than itraconazole base (Fig. 4). The mean cumulative percent releases (±SD) at 24 h were found to be 53.878 ± 1.9, 42.977 ± 1.62, and 64.38 ± 0.98 from capsules containing **1a**, **1b**, and **1c**, respectively. As expected this was noticeably better than itraconazole capsules, which showed cumulative percent release value (±SD) of mere 8.38 ± 0.042. The enhanced dissolution rates of itraconazolium salts can be attributed mainly due to their increased wettability and aqueous solubility. The dissolution efficiency revealed a statistically significant enhancement ($p < 0.05$) in *in vitro* release rate between itraconazole base and itraconazolium salts.

To conclude, the itraconazolium salts **1a–c** were prepared successfully, and as anticipated, they provided substantially higher solubility and dissolution profile than the itraconazole. The reason for amplification in solubility and dissolution profile may be explicated on the basis of increased polar character of the itraconazole molecule after salt formation. Reduced particle size and crystallinity may be counted as other reasons. As the itraconazole is a BCS class II drug, its bioavailability is dissolution rate limited [12,13]. Therefore, itraconazolium salts may provide excellent tools for achieving improved bioavailability.

EXPERIMENTAL

General. Compounds were checked for their purity on precoated silica gel G_{254} TLC plates (Merck KGaA (Darmstadt, Germany)), and the spots were visualized under UV light (254 nm) and then by exposing them to iodine vapors. Quantitative analysis of itraconazole as well as itraconazole salts was performed using UV Spectrophotometer (Thermo Spectronic, Genesys 6 (Thermo Fisher Scientific Inc., Waltham, MA)). Melting points were recorded on capillary melting point apparatus (DB-3135H (Decibel Dynamics Ltd. Chandigarh, India)). Optical micrographs were recorded at 100× magnification by optical microscope fitted with built-in camera (Eclipse 80i, Nikon Instruments Inc., Tokyo, Japan). NMR spectra were recorded on 400 MHz Bruker FT-NMR spectrometer (Bruker India Scientific Pvt. Ltd., New Delhi, India) using tetramethylsilane as internal standard, and the chemical shifts are reported in δ units. Mass spectra were recorded in APCI mode on Finnigan MAT LCQ spectrometer using Xcalibur software (Thermo Electron Corp., Waltham, MA).

General procedure for synthesis of itraconazolium salts. To a stirred solution of itraconazole (5 g, 7.09 mmol) in chloroform (10 mL), a 50% (w/w) methanolic solution of acid (Table 1) was added drop by drop for 5 min, and reaction was allowed to accomplish under reflux conditions by visualizing the depletion of starting material, itraconazole (Table 2). The reaction mixture was washed with water (3×10 mL) to remove the unreacted acid and then dried over Na_2SO_4. Evaporation of chloroform under reduced pressure gave

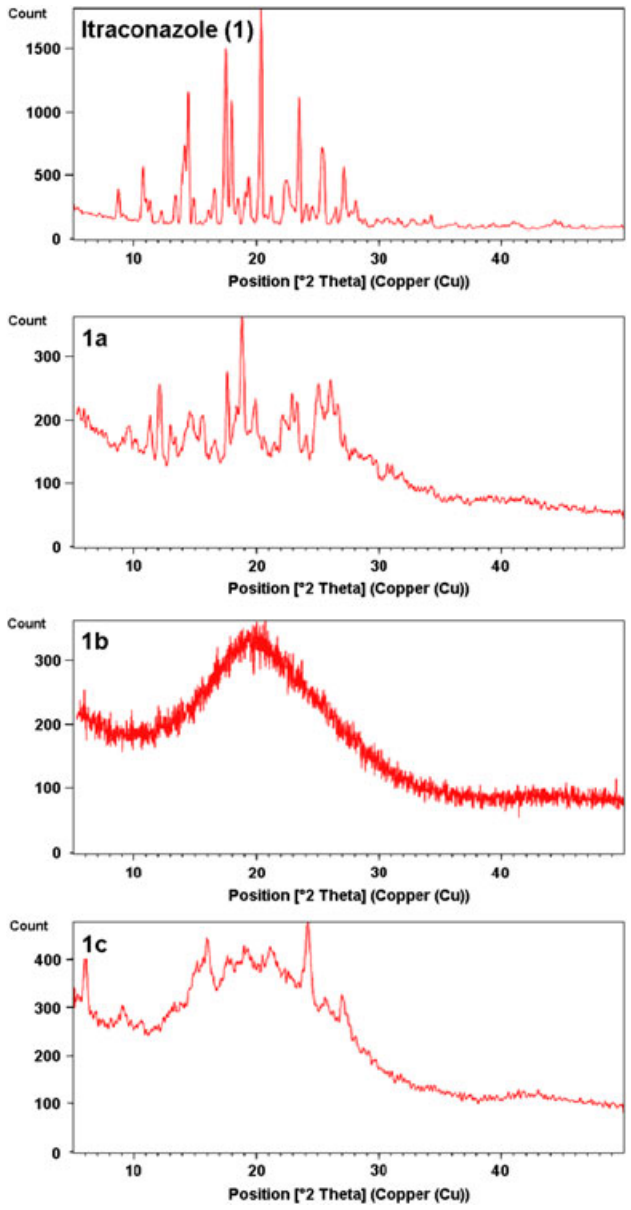


Figure 2. Powder X-ray diffractograms of itraconazole (**1**) and itraconazolium salts **1a–c**. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

white and pale white residues, which were reprecipitated from methanolic solution by addition of water to give itraconazolium salts upon drying under vacuum.

Itraconazolium dinitrate (1a). Yield 93%; pale white solid; mp 118–120°C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89–0.93 (t, 3H, CH_3, J = 7.44 Hz), 1.39–1.41 (d, 3H, CH_3, J = 6.76 Hz), 1.71–1.93 (m, 2H, CH_2), 3.23–3.25 (d, 4H, piperazine CH_2, J = 10), 3.36–3.38 (d, 4H, piperazine CH_2, J = 9.92), 3.48–3.52 (m, 1H, CH), 3.79–3.83 (m, 2H, dioxolane CH_2), 3.91–3.95 (m, 1H, dioxolane CH), 4.27–4.40 (m, 2H, N-CH_2), 4.75–4.87 (m, 2H, O-CH_2), 6.80–6.82 (d, 2H, 2× Ar-H, J = 6.8 Hz), 6.94–6.96 (d, 2H, 2× Ar-H, J = 9.04 Hz), 7.03–7.05 (d, 2H, 2× Ar-H, J = 9.04 Hz), 7.41–7.43 (d, 2H, 2× Ar-H, J = 5.44 Hz), 7.48–7.50 (d, 1H, Ar-H, J = 6.36 Hz), 7.57–7.59 (d, 1H, Ar-H, J = 8. 44 Hz), 7.64 (s, 1H, triazolone CH), 7.90 (s, 1H, triazole CH), 8.21 (s, 1H, triazole CH). MS (APCI): 705.6 (M + 1). *Anal*. Calcd for $C_{35}H_{40}Cl_2N_{10}O_{10}$: C, 50.55; H, 4.85; Cl, 8.53; N, 16.84; O, 19.24. Found: C, 50.34; H, 5.11; Cl, 8.35; N, 16.63; O, 19.60.

Itraconazolium sulfate (1b). Yield 90%; white solid; mp 161–163°C (with decomposition above 164°C); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88–0.92 (t, 3H, CH_3, J = 7.40 Hz), 1.38–1.40 (d, 3H, CH_3, J = 6.72 Hz), 1.60–1.92 (m, 2H, CH_2), 3.22–3.25 (d, 4H, piperazine CH_2, J = 9.96), 3.35–3.38 (d, 4H, piperazine CH_2, J = 10), 3.47–3.51 (m, 1H, CH), 3.78–3.83 (m, 2H, dioxolane CH_2), 3.90–3.94 (m, 1H, dioxolane CH), 4.25–4.39 (m, 2H, N-CH_2), 4.74–4.86 (m, 2H, O-CH_2), 6.79–6.81 (d, 2H, 2× Ar-H, J = 10.88 Hz), 6.93–6.95 (d, 2H, 2× Ar-H, J = 9.08 Hz), 7.03–7.05 (d, 2H, 2× Ar-H, J = 9.04 Hz), 7.41–7.43 (d, 2H, 2× Ar-H, J = 6.96 Hz), 7.47–7.48 (d, 1H, Ar-H, J = 6.06 Hz), 7.56–7.58 (d, 1H, Ar-H, J = 8. 44 Hz), 7.61 (s, 1H, triazolone CH), 7.89 (s, 1H, triazole CH), 8.20 (s, 1H, triazole CH). MS (APCI): 705.6 (M + 1). *Anal*. Calcd for $C_{35}H_{40}Cl_2N_8O_8S$: C, 52.30; H, 5.02; Cl, 8.82; N, 13.94; O, 15.93; S, 3.99. Found: C, 52.61; H, 4.93; Cl, 8.69; N, 14.14; O, 16.10; S, 4.25.

Itraconazolium ditoluenesulfonate (1c). Yield 88%; white solid; mp 141–143°C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89–0.92 (t, 3H, CH_3, J = 7.40 Hz), 1.39–1.40 (d, 3H, CH_3, J = 6.68 Hz), 1.69–1.90 (m, 2H, CH_2), 2.35 (s, 6H, 2× CH_3, Ar-CH_3), 3.78–3.82 (m, 2H, dioxolane CH_2), 3.90–3.93 (m, 11H, O-CH_2, 4× CH_2, dioxolane CH), 4.25–4.38 (m, 3H, N-CH_2), 4.88 (s, 2H, 2× OH), 6.98–7.00 (d, 2H, Ar-H, J = 8.84 Hz), 7.17–7.19 (d, 4H, 4× Ar-H, J = 7.92 Hz), 7.26–7.28 (d, 2H, 2× Ar-H, J = 9.00 Hz), 7.48 (s, 1H, triazolone CH), 7.54–7.60 (m, 3H, 3× Ar-H), 7.66 (s, 1H, Ar-H), 7.78–7.83 (m, 6H, 6× Ar-H), 8.22 (s, 1H, triazole CH), 9.13 (s, 1H, triazole CH); MS (APCI): 705.6 (M + 1). *Anal*. Calcd for $C_{49}H_{52}Cl_2N_8O_{10}S_2$: C, 56.16; H, 5.00; Cl, 6.77; N, 10.69; O, 15.27; S, 6.12. Found: C, 55.87; H, 5.20; Cl, 6.54; N, 10.77; O, 15.38; S, 5.92.

Spectrophotometric method of analysis for 1a–c in different solvents. Various standards (2, 4, 8, 10, 15, 20, and 25 μg/mL) were prepared from a 100 μg/mL stock solution of itraconazolium salts **1a–c** in ethanol (λ_{max} 263 nm) and simulated gastric fluid (SGF) without pepsin, pH 1.2 (λ_{max} 254 nm). These standards were subsequently used to prepare calibration curves of the salts. The method was validated with respect to linearity, accuracy, and precision.

Solubility studies. The solubility of itraconazole salts was determined in water, SGF, and pharmaceutically acceptable polar solvents such as ethanol and propylene glycol. An excess amount of drug and drug salts **1a–c** was added to 5 mL of the solvent taken in a 25 mL conical flask and shaken horizontally in a shaker bath at 37 ± 1°C for 72 h. Subsequently, the samples were filtered through 0.45 μm membrane filter, suitably diluted and analyzed spectrophotometrically.

***In vitro* dissolution study.** Accurately weighed 100 mg of the itraconazole and 100 mg drug equivalent of itraconazolium salts **1a–c** were filled in capsules and were used for the drug release studies. USP dissolution apparatus type II (LabIndia DS 8000, LabIndia Ltd., Mumbai, India) was used for the studies at 100 rpm and 37 ± 0.2°C temperature. The drug release study was carried out in 900 mL of SGF (without pepsin, pH 1.2), and 5 mL aliquots of the solution were withdrawn at predetermined time intervals, replaced by fresh dissolution medium. The samples were analyzed for drug

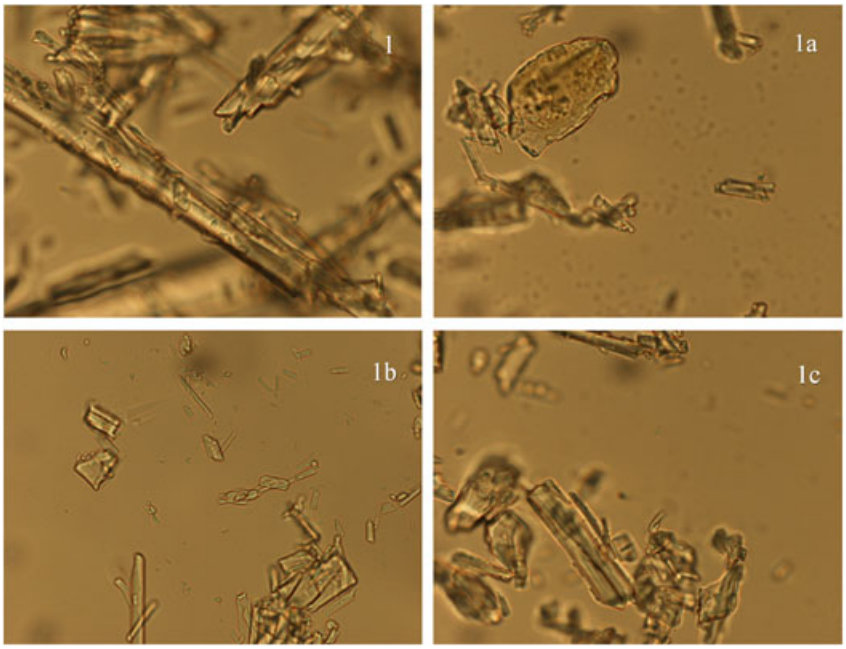


Figure 3. Optical micrographs (100×) of itraconazole (**1**) and itraconazolium salts **1a–c**. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 1

Solubility of itraconazole and itraconazolium salts **1a–c** in various polar solvents.

	Solubility expressed as mg/mL, $n = 3$ (mean ± SD)			
Solvents	Itraconazole	**1a**	**1b**	**1c**
Water	1.00×10^{-6}	$(23.83 \pm 0.134) \times 10^{-3}$	$(22.62 \pm 0.001) \times 10^{-3}$	$(57.8 \pm 0.003) \times 10^{-3}$
Simulated gastric fluid	12.03 ± 0.732	62.03 ± 0.133	55.98 ± 0.876	78.39 ± 0.562
Ethanol	0.04 ± 0.001	12.12 ± 0.134	11.01 ± 0.117	31.96 ± 0.363
Propylene glycol	2.95 ± 0.092	23.77 ± 0.402	22.17 ± 0.351	93.09 ± 0.444

Value of aqueous solubility of itraconazole was obtained from the literature [5].

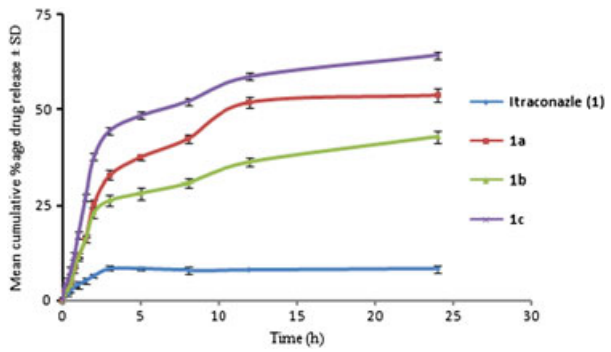


Figure 4. Release profile of itraconazole (**1**) and itraconazolium salts **1a–c** in simulated gastric fluid without pepsin (pH 1.2) at 37 ± 0.5°C ($n = 3$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 2

Experimental conditions for synthesizing itraconazolium salts.

Acid used	Acid taken (in mmol) for 7.09 mmol of itraconazole	Reaction time (min)	Yield (%) of recrystallized itraconazolium salt
HNO_3	14.88	10	93
H_2SO_4	7.79	15	90
$CH_3C_6H_4SO_3H$	14.88	25	88

content using UV spectrophotometer (λ_{max} 254 nm), against dissolution medium taken as blank. The dissolution studies were conducted in triplicate, and the average drug release ± SD was calculated. The dissolution profiles for **1a–c** were compared against dissolution data of itraconazole (taken as control) employing Dunnet's test using a statistical software (SigmaStat 3.5 (Systat Software Inc., Chicago)) for significant difference at 5% confidence limit.

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REFERENCES AND NOTES

[1] Heeres, J.; Meerpoel, L.; Lewi, P. Molecules 2010, 15, 4129.

[2] DiNunzio, J. C.; Brough, C.; Miller, D. A.; Williams, R. O.; McGinity, J. W. J Pharm Sci 2010, 99, 1239.

[3] Moneghini, M.; De Zordi, N.; Solinas, D.; Macchiavelli, S.; Princivalle, F. Future Med Chem 2010, 2, 237.

[4] Lee, S. Y.; Jung, I. I.; Kim, J. K.; Lim, G. B.; Ryu J. H. J Supercritical Fluids 2008, 44, 400.

[5] Peeters, J.; Neeskens, P.; Tollenaere, J. P.; Van Remoortere, P.; Brewster, M. E. J Pharm Sci 2002, 91, 1414.

[6] Remenar, J. F.; Morissette, S. L.; Peterson, M. L.; Moulton, B.; MacPhee J. M.; Guzman, H. R.; Almarsson, O. J Am Chem Soc 2003, 125, 8456.

[7] Tao, T.; Zhao, Y.; Wu, J.; Zhou, B. Int J Pharm 2009, 367, 109.

[8] Miyazaki, S.; Oshiba, M.; Nadai, T. J Pharm Sci 1981, 70, 594.

[9] Thomas, E.; Rubino, J. Int J Pharm 1996, 130, 179.

[10] Jung, J. Y.; Yoo, S. D.; Lee, S. H.; Kim, K. H.; Yoon, D. S.; Lee, K. H. Int J Pharm 1999, 187, 209.

[11] Chen, W.; Gu, B.; Wang, H.; Pan, J.; Lu, W.; Hou, H. Int J Pharm 2008, 362, 133.

[12] Amidon, G. L.; Lennernas, H.; Shah, V. P.; Crison, J. R. Pharm Res 1995, 12, 413.

[13] Swaminathan, S.; Vavia, P. R.; Trotta, F.; Torne, S. J Incl Phenom Macrocycl Chem 2007, 57, 89.