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ScienceDirect
Journal of Fluorine Chemistry 128 (2007) 224–231
JOURNAL OF FLUORINE CHEMISTRY
www.elsevier.com/locate/fluor

Short communication

Synthesis of biologically important novel fluorinated spiro heterocycles under microwaves catalyzed by montmorillonite KSF

Kapil Arya [a,*], Anshu Dandia [b]

[a] *Indian Oil Corporation Limited, Catalyst Division, R&D center, Sector-13, Faridabad 121007, Haryana, India*
[b] *Chemistry Department, University of Rajasthan, Jaipur 302004, India*

Received 5 November 2006; received in revised form 23 November 2006; accepted 3 December 2006
Available online 6 December 2006

Abstract

The arylidienes of fluorinated spiro thiazolidines (**5**) containing α,β-unsaturated function have been used as component of Micheal addition with equimolar amount of 2-aminopyridine (**6a**) to give novel fluorinated spiro [indole-3,2′-pyrido[1,2-a]thiazolo[5,4-e]pyrimidines] (**7**) in a single step under microwaves in presence of montmorillonite KSF as solid support. The new improved synthetic method for fluorinated spiro [indole-3,2′-thiazolo[4,5-d]pyrimidines] (**8**) has also been developed involving the reaction of (**5**) with thiourea under monomode microwave reactor. Comparison with conventional synthesis and multimode microwave oven indicated the enhanced yield with faster reactions under monomode microwave reactor. Structure–activity relationships between the chemical structures and the antimycobacterial, antifungal activity of the evaluated compounds are also discussed.

Keywords: Fluorinated spiro thiazolidines; MW irradiation; Arylidenes derivatives; Thiazolopyrimidines; Monomode microwave reactor

1. Introduction

The chemistry of spiro indoles in which an indole ring is joined to sulfur and nitrogen containing heterocycles at the C-3 position through a spiro carbon atom is of great interest due to their physiological and biological activities [1,2]. Spiro [indole-thiazolidines] are known to possess various biological activity, e.g. anti-inflammatory [3], antimicrobial [4], bacteriostatic [5] and anticonvulsant activities [6] and antifungal agents [7]. The significance of these compounds can be judged from the fact that most of the references of spiro-indoles in the literature are patents [8]. Arylidene derivatives of condensed 4-thiazolidinones have been found to be better medicinal agents than the parent 4-thiazolidinones. Besides, condensed 4-thiazolidinones are better anti-HIV, anti-anticonvulsant [9], anti-inflammatory [10] and Hypnotic agents [11] than their thiazole counterparts.

Incorporation of fluorine to different heterocycles is known to affect the course of the reaction besides, influencing the biological/pharmacological activity [12]. Further, incorporation of a trifluoromethyl substituents have been shown to increase the phytoxicity along with selectivity and arenes bearing a trifluoromethyl substituents comprise the largest subgroup of commercially promising pesticides and herbicides. For the preparation of the complex molecules, large efforts have been directed towards the synthetic manipulation of triazolopyrimidines. As a result, scanty reports have appeared which usually require drastic conditions, prolonged reaction times, complex synthetic pathways and often react in organic solvents [13,14]. Thus, new route for the synthesis of these molecules have attracted considerable attention in search for a rapid entry to these heterocycles.

Microwave accelerated synthesis is also emerging as a powerful tool for high-throughput organic synthesis [15,16]. It has been demonstrated that the use of microwave heating can dramatically shorten reaction times, increase product purities and yields, and allow precise control of reaction parameters.

Microwaves also have shown an advantage where processes involve sensitive reagents or when products may decompose under prolonged reaction conditions. In view of this, more interest has now been focused on dry media synthesis,

* Corresponding author.
E-mail address: aryakapil2001@yahoo.com (K. Arya).

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doi:10.1016/j.jfluchem.2006.12.003

involving the coupling of MWI with solid supported reagents. The method provides unique chemical process with special attribute to greater selectivity and ease of manipulation.

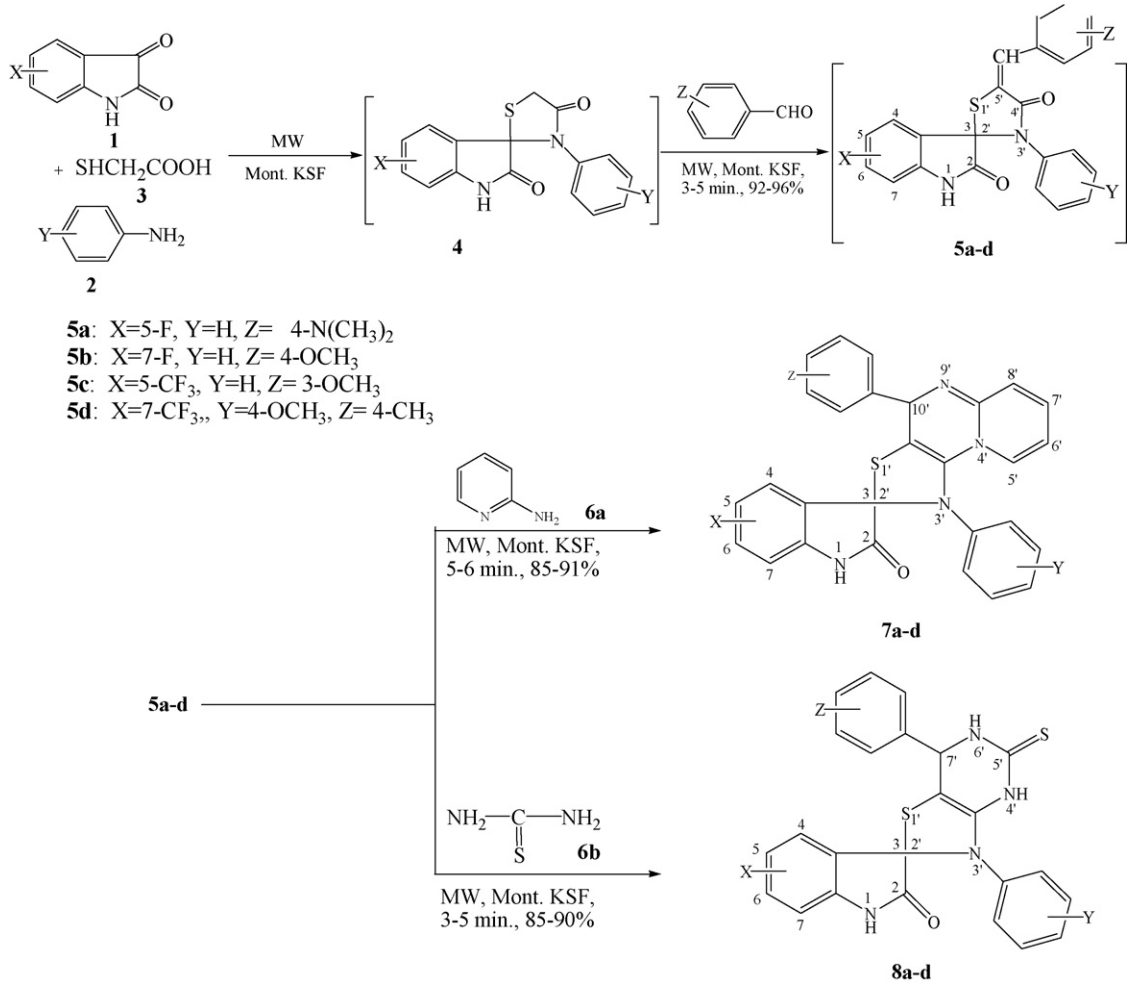
Literature survey reveals that there is no report on the synthesis of titled novel nucleus yet so far. Therefore, it was thought that it would be desirable to develop a facile, efficient, environeconomic, microwave induced method for preparation of novel fluorinated spiro [indole-3,2′-pyrido[1,2-a]thiazolo[5,4-e] pyrimidines] (**7**) and fluorinated 5′-thioxo-4,5,6,7-tetrahydro-2H,3′H-spiro [indole-3,2′-thiazolo[4,5-d] pyrimidines] by the reaction of arylidiens derivative (**5**) with 2-aminopyridine (**6a**) and thiourea (**6b**) using montmorillonte KSF as solid support under microwaves.

As part of our ongoing program to develop efficient and robust methods for the preparation of biologically relevant compounds [17] from readily available building blocks, which are novel yet resemble known biologically active molecules by virtue of the presence of some critical structural features, we sought to develop a convenient preparation of a novel fluorinated spiro indolines **7** and **8** (Scheme 1) by combining two to three steps as a one-pot reaction to reduce the pollution at source as important green chemical theme [18] under microwaves.

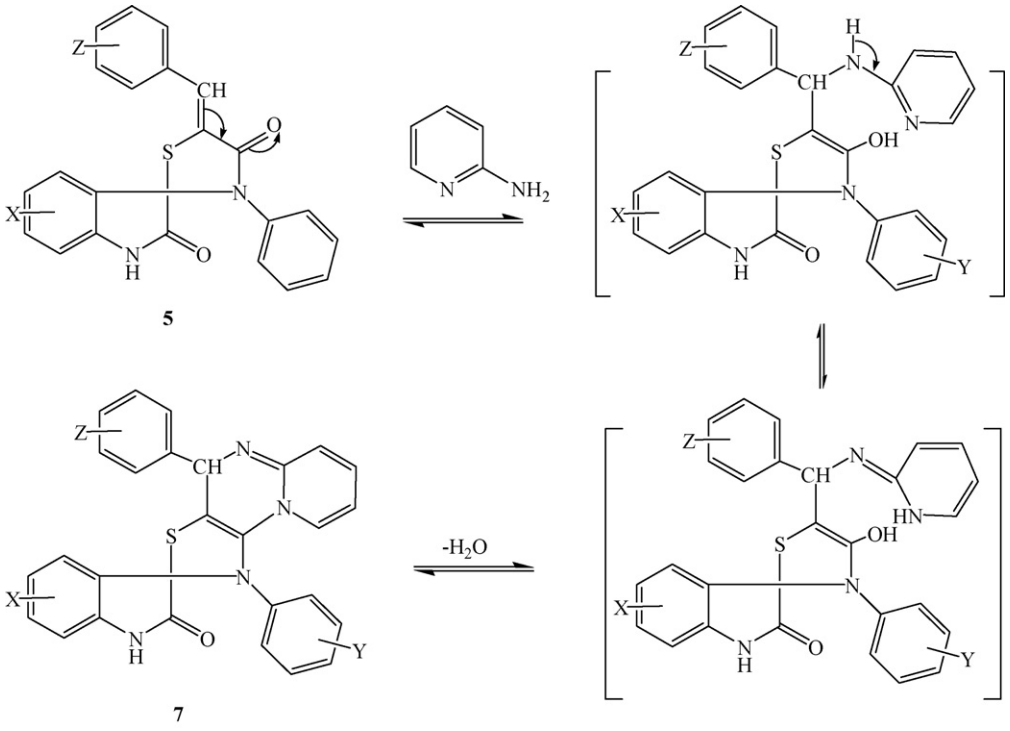
2. Results and discussion

Fluorinated spiro [indole-thiazolidines] (**4**) were prepared by the multicomponent condensation between indole-2,3-dione,amines and mercaptoacetic acid [19] using montmorillonite KSF as solid support. These on reaction with arylaldehydes "in situ" by Knoevengal condensation yielded 3′aryl-5′-benzylidene-4′*H*-spiro-[indole-3,2′-thiazolidine]-2,4′(1*H*)-dione (**5a–d**) in 92–96% yield in 3–5 min under same reaction conditions in one pot. These arylidenes with an α, β-unsaturated ketonic function (–CH=CH–CO–) in their structure have been used as a component of Micheal addition with 2-aminopyridine and yielded the novel compound fluorinated spiro [indole-3,2′-pyrido[1,2-a]thiazolo[5,4-e] pyrimidines]-2 (1*H*)-one (**7**) in a single step in presence of montmorillonite KSF as solid support in 85–91% yield in 5–6 min. A non-isolated intermediate has been presumed during such type of condensation (Scheme 2).

Further fluorinated 5′-thioxo-4,5,6,7-tetrahydro-spiro [indole-3,2′-thiazolo[4,5-d]pyrimidine]-2(1*H*)-one (**8a–d**) were also synthesized by improved enviroeconomic green chemical procedure by the reaction of arylidenes derivatives (**5**) with thiourea in 85–90% yield in 3–5 min.



Scheme 1.



Scheme 2.

Conventional synthesis of **5**, **7** and **8** requires long reflux period in volatile solvents such as dioxane, dry toluene, glacial AcOH and fused sodium acetate and using Dean Strak apparatus make whole procedure very tedious and yielding the desired product in very low quantity.

The irradiation was performed in parallel using two kinds of reactors: a usual microwave multimode reactor (National panasonic oven) having 1000 W power, in this case the microwaves are not focused, and a monomode reactor having 50 W power (Maxidigest MY-350 Prolabo) with focused rays and a much more homogenous electromagnetic field. In comparison, the reactions realized under microwave irradiation and interphase catalysis had higher yields than those performed under classical conditions for the same time and temperature.

From the results shown in Table 1, it can be seen than for the same time at the same temperature, the reactions performed under microwave irradiation, using both the multimode reactor at 1000 W and the monomode reactor, were faster than the reaction performed by classical heating. Same yields were obtained using both reactors but for different time intervals.

The structure assigned to arylidiene derivatives (**5**) was confirmed by spectral studies. ^{1}H NMR spectrum of **5a** showed characteristic signals at δ 2.49 (s, 3H, CH_3), 2.79 (s, 3H, CH_3), 5.1 (s, 1H, CH) and 7.13–7.64 (m, 12H, Ar–H) and 9.23 (s, 1H, NH). Absence of CH_2 signal (dd) in ^{1}H NMR at δ 3.80–4.22 ppm further confirmed the formation of **5**. In ^{13}C NMR spectrum of **5** signals were observed at δ 81.6 (spiro carbon), 113.2–147.3 (olefinic carbon and aromatic carbons), 162.8, 169.2 (both C=O).

Table 1
Comparative study of Micheal addition reaction on solid support mont. KSF

Compound no.	X	Y	Z	Rxn temp. (°C)	Reaction time (min)/yield (%)		
					Δ	MW	
						Multimode	Monomode
5a	5-F	H	4-$N(CH_3)_2$	120	300/58	6/92	5/92
5b	7-F	H	4-OCH_3	120	320/54	5/94	4/94
5c	5-CF_3	H	3-OCH_3	120	280/60	8/96	5/96
5d	7-CF_3	4-OCH_3	CH_3	120	340/61	6/95	3/95
7a	5-F	H	4-$N(CH_3)_2$	100	480/54	9/89	5/89
7b	7-F	H	4-OCH_3	100	500/55	8/91	5/91
7c	5-CF_3	H	3-OCH_3	100	480/52	11/90	6/90
7d	7-CF_3	4-OCH_3	CH_3	100	520/58	9/85	5/85
8a	5-F	H	4-$N(CH_3)_2$	100	540/62	8/86	4/86
8b	7-F	H	4-OCH_3	100	480/59	9/90	5/90
8c	5-CF_3	H	3-OCH_3	100	620/60	8/88	4/89
8d	7-CF_3	4-OCH_3	CH_3	100	560/53	9/85	3/85

The formation of **7**and **8** from arylidene derivatives (**5**) was confirmed by IR and ^{1}H NMR data. In IR spectrum of **7a** and **8a** disappearance of C=O absorption band at 1680–1695 cm^{-1} which was present in **5** confirmed the cyclization or involvement of α,β-unsaturated carbonyl system. ^{1}H NMR spectrum of **7a** showed characteristic signals at δ 3.71 (s, 1H, C–10′H), 5.91 (m, 1H, C–8′H), 7.02 (m, 1H, C–6′H), 7.23–8.02 (m, 16H, Ar–H) and 9.01 (s, 1H, NH). Formation of **7a** was further confirmed by ^{13}C NMR and Mass spectra. ^{13}C NMR spectrum of **7a** showed signals at δ 58.9 (C-10′), 84.9 (spiro carbon), 107.6–142.6 (aromatic carbons), 165.2 (C=N) and 168.5 (NH–C=O). Mass spectrum of **7a** showed molecular ion peak *m/z* at 505 ([M]$^+$, 14.6%) corresponding to its molecular weight along with base peak at 385 ([M$^+$−C_8H_{10}N], 100%) and other peaks at 281 (12.3), 188 (13), 160 (20.8), 121 (58.2), 78 (19.3).

^{1}H NMR spectrum of **8a** showed characteristic signals at δ 4.82 (s, 1H, C–7′H), 6.98–7.62 (m, 12H, Ar–H), 9.13 (s, 1H, NH) and 10.25 (bs, 2H, pyridine NH). ^{13}C NMR spectrum of **8a** showed signals at δ 52.6 (C-7′), 92.8 (spiro carbon), 112.9–139.6 (aromatic carbons), 169.4 (C=O) and 176.5 (C=S). Mass spectrum of **8a** showed molecular ion peak *m/z* at 487 ([M]$^+$, 17.4%) corresponding to its molecular weight along with base peak at 443 ([M$^+$−CS], 100) and other peaks at 367 ([M$^+$−C_8H_{10}], 25.5), 281 (42), 185 (25), 165 (28.5), 131 (63.7), 78 (50.4). The presence of fluorine was confirmed by ^{19}F NMR spectra, where the C–F signal was observed at δ 118.05–120.42 (compound **5a**,**b**, **7a**,**b**, **8a**,**b**) and CF_3 signal at δ 63.25–64.07 ppm in case of compound **5c**,**d**, **7c**,**d**, **8c**,**d**.

2.1. Evaluation of antimicrobial and antifungal activity

All compounds prepared were evaluated for their in vitro antimycobacterial activity. The highest activity (72% inhibition) against *Mycobacterium tuberculosis* was found for 5-trifluoromethyl-spiro [indole-3,2′-pyrido[1,2-a]thiazolo[5,4-e] pyrimidines] (**7c**) and 5-trifluoromethyl-5′-thioxo-4,5,6,7-tetrahydro-spiro [indole-3,2′-thiazolo[4,5-d]pyrimidine] (**8c**). Two other compounds (**7d**, **8d**) with the identical substitution on the aromatic part of the molecule, exert a comparable activity. The majority of compounds exhibited only modest antimycobacterial activity (see Table 2).

The evaluation of in vitro antifungal activity of the synthesized compounds was performed against eight fungal strains. The results revealed no interesting activity against the majority of strains tested.

Only the 5-fluoro-spiro [indole-3,2′-pyrido[1,2-a]thiazolo[5,4-e] pyrimidines] (**7a**) and 5-fluoro-5′-thioxo-4,5,6,7-tetrahydro-spiro [indole-3,2′-thiazolo[4,5-d]pyrimidine] (**8a**) showed some promising in vitro antifungal activity against *Trichophyton mentagrophytes*, the most susceptible fungal strain evaluated (MIC = 31.24/63.85 μmol ml^{-1}), although this activity is only modest in comparison with fluconazole, the standard (MIC = 3.91 μmol ml^{-1} after 120 h, see Table 2). The negative antifungal screening results do not allow us to draw detailed conclusions on potential structure–activity relationships.

Table 2
Calculated antimycobaterial evaluation (% of inhibition) and antifungal susceptibility (MIC) comparison with standards: pyrazinamide (PZA) and fluconazole

Compound	X	% Inhibition at 6.25 μg ml^{-1}	MIC (μmol ml^{-1})
7a	5-F	50	31.24/63.85
7b	7-F	54	31.23/31.23
7c	5-CF_3	71	125/250
7d	7-CF_3	68	>500/>500
8a	5-F	58	30.23/61.2
8b	7-F	56	>500/>500
8c	5-CF_3	72	>500/>500
8d	7-CF_3	69	>500/>500
PZA	–	100[a]	–
Fluconazole	–	–	1.95/3.91

[a] MIC = 12.5 μg ML^{-1}, data from ref. [20].

3. Conclusion

After studying the reaction of arylidienes of spiro thiazolidines (**5**) containing α,β-unsaturated function and 2-aminopyridine (**6a**) and thiourea (**6b**) in the solid phase and with an interphase transfer catalyst, by classical heating or under microwave irradiation, it can be concluded that reaction performed much more efficient on the solid support compare than those taking place in an organic solvent because: mont. KSF is a very strong acid causing Micheal addition, which, in this case, determines the ''in situ'' formation of the ylides; the solid support allows the reaction temperature to be raised over the boiling temperature of the solvent; the yields of the reactions which are performed under the influence of microwaves, both in the solid phase and with interphase catalysis, are superior to those reactions performed under classical conditions, due to the temperature being more homogeneous and changes in the activation enthalpy and entropy; the monomode reactor is more efficient than the multimode reactor due to the better yield of energy obtained by microwave fascicle focalization and the more homogeneous electromagnetic field.

On the basis of the biological evaluation, compounds **7c**, **8c** seems to be very attractive as a antimicrobial agents, while, among the compounds bearing trifluoromethyl (**7c–d**, **8c–d**) have shown the highest antituberculosis activity, i.e. against *M. tuberculosis* H37Rv.

4. Experimental

Melting points were determined in open glass capillaries and were uncorrected. Thin layer chromatography on silica gel 'G' coated glass plates using benzene, ethanol (8:2) as eluent was used for monitoring the progress of the reactions. IR spectra (KBr) were recorded on a Magna FT IR–550 spectrophotometer, ^{1}H, ^{19}F and ^{13}C NMR spectra [$CDCl_3$] were taken on a Bruker-300DX spectrometer at 300 and 200 MHz, respectively, using TMS as an internal standard for PMR and hexafluorobenzene as external standard for ^{19}F NMR and mass spectra were recorded on Jeol D-300 spectrometer at an ionisation potential of 70 eV. Microwave assisted reactions

were carried out on a BPL BMO model, operating at 700 W, generating 2450 MHz frequency. All anilines were purchased from Aldrich Chemical Co. and were used as received.

Microwave assisted reactions were carried out on a National panasonic oven with inverter technology operating at fixed frequency 2450 MHz with power out put range 1000 W. For the monomode reactor, Maxidigest MX 350 Prolabo (50 W) were used. Perkin-Elmer 2400 automatic CHNS analyzer used for elemental analyses.

4.1. Fluorinated spiro [indole-3,2′-thiazolidine]-2,4′ (1H)-dione (4)

These compounds were synthesized in one pot by multicomponent cyclocondensation reaction of Fluorinated indole-2,3-dione, amines and mercaptoacetic acid using montmorillonite KSF as solid support as reported by us [19]. Since TLC studies showed 100% conversion with formation of single product hence it is used as such for further conversion without isolating them. For structure confirmation some products are isolated by desorption with methanol and compared with authentic samples prepared by literature methods [21].

4.2. 5′-(4-N,N-dimethylbenzylidene)-3′-phenyl-5-fluoro-2′H-spiro[indole-3,2′-thiazolidine]-2,4′(1H)-dione (5a)

It was synthesized by following different ways.

4.2.1. Conventional method

An equimolar mixture of **4a** (2.75 g, 0.01 mol), *N*,*N*-dimethylbenzaldehyde (1.75 g, 0.01 mol) and anhydrous sodium acetate in glacial acetic acid (40 ml) was refluxed for 5 h. On cooling, the reaction mixture was poured into ice-cold water. The solid thus obtained was filtered, washed with water, dried and crystallized from benzene.

4.2.2. Microwave mediated synthesis

The *N*,*N*-dimethylbenzaldehyde (1.75 g, 0.01 mol) and fluorinated spiro thiazolidinone (**4a**) (synthesized ''in situ'') were dissolved in 50 ml anhydrous benzene. To this solution, 10 g of mineral support (mont KSF) was added under stirring. After solvent evaporation under low pressure, the obtained solid was exposed to microwaves. The activated solid was cooled and washed several times with 10 ml of benzene. Then, the solvent was evaporated and the product purified by methanol recrystallization.

Yield: 92%; mp 240–242 °C; *Rf* 0.6 (petroleum ether, EtOAc, 80:20).

IR (KBr): 3380–3290 (bs, NH), 1720, 1680 (both C=O) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 2.49$ (s, 3H, CH_3), 2.79 (s, 3H, CH_3), 5.10 (s, 1H, CH), 7.13–7.64 (m, 12H, Ar–H), 9.23 (s, 1H, NH); ^{19}F NMR ($CDCl_3$): $\delta = -118.09$ (s, F); ^{13}C NMR ($CDCl_3$): $\delta = 81.6$ (spiro carbon), 113.2–147.3 (olefinic carbon and Ar carbons), 162.8, 169.2 (both C=O); Anal. Calcd for $C_{25}H_{21}N_3O_2S$: C, 70.23; H, 4.95; N, 9.83. Found: C, 70.39; H, 4.89; N, 9.78.

4.3. 5′-(4-Methoxybenzylidene)-3′-phenyl-7-fluoro-2′H-spiro[indole-3,2′-thiazolidine]-2,4′(1H)-dione (5b) [22]

Yield: 94%; mp 205–207 °C; *Rf* 0.7 petroleum ether, EtOAc, 80:20).

IR (KBr): 3370–3250 (bs, NH), 1715, 1690 (both C=O), 1150 (C–O–C), 760 (C–Cl) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 3.62$ (s, 3H, OCH_3), 5.21 (s, 1H, CH), 7.05–7.68 (m, 12H, Ar–H), 9.28 (s, 1H, NH); ^{19}F NMR ($CDCl_3$): $\delta = -119.23$ (s, F); Anal. Calcd for $C_{24}H_{17}ClN_2O_3S$: C, 64.21; H, 3.82; N, 6.24. Found: C, 64.15; H, 3.92; N, 6.35.

4.4. 5′-(3-Methoxybenzylidene)-3′-phenyl-5-trifluoromethyl-2′H-spiro[indole-3,2′-thiazolidine]-2,4′(1H)-dione 5c

Yield: 96%; mp 215–218 °C; *Rf* 0.6 petroleum ether, EtOAc, 80:20).

IR (KBr): 3360–3290 (bs, NH), 1720, 1695 (both C=O), 1150 (C–O–C) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 3.57$ (s, 3H, OCH_3), 5.4 (s, 1H, CH), 7.10–7.85 (m, 12H, Ar–H), 9.36 (s, 1H, NH); ^{19}F NMR ($CDCl_3$): $\delta = -63.89$ (s, CF_3); Anal. Calcd for $C_{25}H_{20}N_2O_3S$: C, 70.07; H, 4.70; N, 6.54. Found: C, 70.15; H, 4.62; N, 6.45.

4.5. 5′-(4-Methylbenzylidene)-3′-(4-methoxyphenyl)-7-trifluoromethyl-2′H-spiro[indole-3,2′-thiazoli-dine]-2,4′(1H)-dione 5d

Yield: 95%; mp 192–195; *Rf* 0.9 petroleum ether, EtOAc, 80:20).

IR (KBr): 3390–3280 (bs, NH), 1710, 1690 (both C=O), 1140 (C–O–C) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 2.28$ (s, 3H, CH_3), 3.58 (s, 3H, OCH_3), 5.18 (s, 1H, CH), 7.15–7.89 (m, 11ssssH, Ar–H), 9.23 (s, 1H, NH); ^{19}F NMR ($CDCl_3$): $\delta = -64.23$ (s, CF_3); Anal. Calcd for $C_{25}H_{20}N_2O_3S$: C, 70.18; H, 4.70; N, 6.54. Found: C, 70.18; H, 4.62; N, 6.48.

4.6. 10′-(4-N,N-dimethylphenyl)-3′phenyl-5-fluoro-spiro[indole-3,2′-pyrido[1,2-a]thiazolo[5,4-e] pyrimi-dines] (7a)

It was synthesized by following methods.

4.6.1. Conventional method

(i) An equimolar mixture of **5a** (4.27 g, 0.01 mol), 2-amino pyridine (**6a**) (0.94 g, 0.01 mol) and fused anhydrous sodium acetate (2 g) in dioxane (40 ml) was refluxed for 8 h. The solvent was removed by distillation in vacuo and residue poured into cold water. The solid thus obtained was washed with water and crystallized from ethanol to give **7a**. mp = 132 °C, yield = 54%.

(ii) A equimolar mixture of **5a** (4.27 g, 0.01 mol), 2-amino pyridine (**6a**) (0.94 g, 0.01 mol) in ethanol (30 ml) containing six to eight drops of glacial acetic acid was refluxed for 7 h. A crude product appeared on cooling the reaction mixture, which was filtered, washed with water

and recrystallized form ethanol to give desired product **7a**. mp = 132 °C, yield = 57%.

4.6.2. Microwave mediated synthesis

An equimolar mixture (0.01 mol) of **5a** and **6a** was adsorbed on montmorillonite KSF (70% weight of reactants). The reaction mixture was irradiated for appropriate time (Table 1). The recyclable montmorillonite KSF separated by eluting the product with methanol and excess solvent was evaporated on rotaevaporator to give pure product (TLC), with no need of further purification.

7a: Yield: 89%; mp 132–135 °C; *Rf* 0.6 (petroleum ether, EtOAc, 80:20).

IR (KBr): 3380–3270 (bs, NH), 1710 (C=O), 1620 (C=N) cm^{-1};

^1H NMR ($CDCl_3$): δ = 2.45 (s, 3H, CH_3), 2.73 (s, 3H, CH_3), 3.71 (s, 1H, C–10′H), 5.91 (m, 1H, C–8′H), 7.02 (m, 1H, C–6′H), 7.23–8.02 (m, 16H, Ar–H), 9.01 (s, 1H, NH); ^{19}F NMR ($CDCl_3$): δ = −118.54 (s, F); ^{13}C NMR (DMSO-d_6): δ = 58.9 (C-10′), 84.9 (spiro carbon), 107.6–142.6 (aromatic carbons), 165.2 (C=N), 168.5 (NH–C=O); GC–MS (EI): *m/z* (%) = 505 [M^+], 385 (100) [$M^+-C_8H_{10}N$], 281 (12.3), 188 (13), 160 (20.8), 121 (58.2), 78 (19.3); Anal. Calcd for $C_{30}H_{27}N_5O_5$: C, 71.26; H, 5.38; N, 13.85. Found: C, 71.19; H, 5.31; N, 13.92.

4.7. 10′-(4-methoxy)-3′phenyl-7-fluoro-spiro[indole-3,2′-pyrido[1,2-a]thiazolo[5,4-e] pyrimidines] ***7b***

Yield: 91%; mp 137–139 °C; *Rf* 0.6 (petroleum ether, EtOAc, 80:20).

IR (KBr): 3360–3290 (bs, NH), 1720 (C=O), 1610 (C=N), 1150 (C–O–C) cm^{-1}; ^1H NMR ($CDCl_3$): δ = 3.62 (s, 3H, OCH_3), 3.81 (s, 1H, C–10′H), 5.87 (m, 1H, C–8′H), 7.05 (m, 1H, C–6′H), 7.18–8.09 (m, 16H, Ar–H), 9.15 (s, 1H, NH); ^{19}F NMR ($CDCl_3$): δ = −119.56 (s, F); Anal. Calcd for $C_{29}H_{23}ClN_4O_2S$: C, 66.09; H, 4.40; N, 10.63. Found: C, 66.21; H, 4.51; N, 10.71.

4.8. 10′-(3-methoxy)-3′phenyl-5-trifluoromethyl-spiro[indole-3,2′-pyrido[1,2-a]thiazolo[5,4-e] pyrimidines] ***7c***

Yield: 90%; mp 148–150 °C; *Rf* 0.8 (petroleum ether, EtOAc, 80:20).

IR (KBr): 3375–3290 (bs, NH), 1715 (C=O), 1620 (C=N), 1160 (C–O–C) cm^{-1}; ^1H NMR ($CDCl_3$): δ = 3.56 (s, 3H, OCH_3), 3.79 (s, 1H, C–10′H), 5.86 (m, 1H, C–8′H), 7.01 (m, 1H, C–6′H), 7.15–8.12 (m, 16H, Ar–H), 9.28 (s, 1H, NH); ^{19}F NMR ($CDCl_3$): δ = −63.47 (s, CF_3); Anal. Calcd for $C_{30}H_{26}N_4O_2S$: C, 71.12; H, 5.17; N, 11.06. Found: C, 71.26; H, 5.02; N, 11.23.

4.9. 10′-(4-methyl)-3′-(4-methoxyphenyl)- 7-trifluoromethyl spiro[indole-3,2′-pyrido[1,2-a]thiazolo[5,4-e]pyrimidines] ***7d***

Yield: 85%; mp 118–120 °C; *Rf* 0.7 (petroleum ether, EtOAc, 80:20).

IR (KBr): 3360–3250 (bs, NH), 1710 (C=O), 1610 (C=N), 1145 (C–O–C) cm^{-1}; ^1H NMR ($CDCl_3$): δ = 2.28 (s, 3H, CH_3), 3.58 (s, 3H, OCH_3), 3.79 (s, 1H, C–10′H), 5.95 (m, 1H, C–8′H), 6.95 (m, 1H, C–6′H), 7.10–8.02 (m, 15H, Ar–H), 9.26 (s, 1H, NH); ^{19}F NMR ($CDCl_3$): δ = −63.25 (s, CF_3); ^{13}C NMR (DMSO-d_6): δ = 57.9 (C-10′), 83.9 (spiro carbon), 106.3–145.6 (aromatic carbons), 165.2 (C=N), 168.5 (NH–C=O); Anal. Calcd for $C_{30}H_{26}N_4O_2S$: C, 71.12; H, 5.17; N, 11.06. Found: C, 71.02; H, 5.07; N, 11.11.

*4.10. 7′-(4-N,N-dimethylphenyl)-3′-phenyl-5-fluoro-5′-thioxo-4,5,6,7-tetrahydro-spiro[indole-3,2′-thiazo-lo[4,5-d]pyrimidine] (**8a**)*

It was synthesized by following different way.

4.10.1. Conventional method

An equimolar mixture of **5a** (2.75 g, 0.01 mol), thiourea (**6b**) (0.78 g, 0.01 mol) in absolute ethanol (25 ml)/anhyd pyridine (25 ml) was refluxed for 9 h. On cooling, the crude solid appeared which was filtered, washed with ice-cold water containing dilute HCl with constant stirring. The solid mass thus obtained was filtered and crystallized from ethanol. mp = 212 °C, yield = 62%.

4.10.2. Microwave mediated synthesis

It was synthesized by reaction of **5a** and **6b** using montmorillonite KSF as solid support under microwaves as similar manner as preparation of **7a**.

Yield: 86%; mp 212–215 °C; *Rf* 0.5 (petroleum ether, EtOAc, 80:20).

IR (KBr): 3380–3280 (bs, NH), 1710 (C=O), 1220 (C=S) cm^{-1}; ^1H NMR ($CDCl_3$): δ = 2.43 (s, 3H, CH_3), 2.85 (s, 3H, CH_3), 4.82 (s, 1H, C–7′H), 6.98–7.62 (m, 12H, Ar–H), 9.13 (s, 1H, NH), 10.25 (bs, 2H, pyridine NH); ^{19}F NMR ($CDCl_3$): δ = −120.36 (s, F); ^{13}C NMR (DMSO-d_6): δ = 52.6 (C-7′), 92.8 (spiro carbon), 112.9–139.6 (aromatic carbons), 169.4 (C=O), 176.5 (C=S); GC–MS (EI): *m/z* (%) = 487 [M^+], 443 (100) [M^+-CS], 367 (25.5) [$M^+-C_8H_{10}$], 281 (42), 185 (25), 165 (28.5), 131 (63.7), 78 (50.4); Anal. Calcd for $C_{26}H_{25}N_5OS_2$: C, 64.04; H, 5.17; N, 14.36. Found: C, 64.15; H, 5.29; N, 14.42.

4.11. 7′-(4-methoxy)-3′-phenyl-7-fluoro-5′-thioxo-4,5,6,7-tetrahydro-spiro[indole-3,2′-thiazolo[4,5-d]pyrimidine] ***8b***

Yield: 90%; mp 170–172 °C; *Rf* 0.6 (petroleum ether, EtOAc, 80:20).

IR (KBr): 3380–3250 (bs, NH), 1720 (C=O), 1230 (C=S), 1145 (C–O–C) cm^{-1}; ^1H NMR ($CDCl_3$): δ = 3.58 (s, 3H, OCH_3), 4.87 (s, 1H, C–7′H), 7.01–7.85 (m, 12H, Ar–H), 9.19 (s, 1H, NH), 10.32 (bs, 2H, pyridine NH); ^{19}F NMR ($CDCl_3$): δ = −119.08 (s, F); Anal. Calcd for $C_{25}H_{21}ClN_4O_2S_2$: C, 58.99; H, 4.16; N, 11.01. Found: C, 58.78; H, 4.08; N, 11.09.

4.12. 7′-(3-methoxy)-3′-phenyl-5-trifluoromethyl-5′-thioxo-4,5,6,7-tetrahydro-spiro[indole-3,2′-thiazo-lo[4,5-d]pyrimidine] **8c**

Yield: 89%; mp 134–136 °C; *Rf* 0.7 (petroleum ether–EtOAc, 80:20).

IR (KBr): 3390–3260 (bs, NH), 1720 (C=O), 1225 (C=S), 1145 (C–O–C) cm^{-1}. ^1H NMR ($CDCl_3$): $\delta = 3.65$ (s, 3H, OCH_3), 4.76 (s, 1H, C–7′H), 7.05–7.89 (m, 12H, Ar–H), 9.25 (s, 1H, NH), 10.18 (bs, 2H, pyridine NH); ^{19}F NMR ($CDCl_3$): $\delta = -64.36$ (s, CF_3); Anal. Calcd for $C_{26}H_{24}N_4O_2S_2$: C, 63.91; H, 4.95; N, 11.47. Found: C, 63.75; H, 4.89; N, 11.56.

4.13. 7′-(4-methyl)-3′-(4-methoxyphenyl)-7-trifluoromethyl 5′-thioxo-4,5,6,7-tetrahydro-spiro[indole-3,2′-thiazolo[4,5-d]pyrimidine] **8d**

Yield: 85%; mp 185–187 °C; *Rf* 0.7 (petroleum ether, EtOAc, 80:20).

IR (KBr): 3395–3260 (bs, NH), 1715 (C=O), 1235 (C=S), 1145 (C–O–C) cm^{-1}. ^1H NMR ($CDCl_3$): $\delta = 2.35$ (s, 3H, CH_3), 3.54 (s, 3H, OCH_3), 4.81 (s, 1H, C–7′H), 6.96–7.89 (m, 11H Ar–H), 9.26 (s, 1H, NH), 10.23 (bs, 2H, pyridine NH); ^{19}F NMR ($CDCl_3$): $\delta = -63.94$ (s, CF_3); ^{13}C NMR (DMSO-d_6): $\delta = 58.2$ (C-10′), 85.3 (spiro carbon), 108.9–142.3 (aromatic carbons), 163.2 (C=N), 166.3 (NH–C=O); Anal. Calcd for $C_{26}H_{24}N_4O_2S_2$: C, 63.91; H, 4.95; N, 11.47. Found: C, 63.84; H, 4.86; N, 11.41.

4.14. In vitro antifungal susceptibility testing

The broth microdilution test [23] was used for the assessment of in vitro antifungal activity of the synthesized compounds against *Candida albicans* ATCC 44859 (CA), *Candida tropicalis* 156 (CT), *Candida krusei* E28 (CK), *Candida glabrata* 20/I (CG), *Trichosporon beigelii* 1188 (TB), *Aspergillus fumigatus* 231 (AF), *Absidia corymbifera* 272 (AC) and *T. mentagrophytes* 445 (TM). Fluconazole was used as a reference drug. The procedure was performed with two-fold dilution of the compounds in RPMI 1640 medium (Sevapharma) buffered to pH 7.0 with 0.165 mol of 3-morpholinopropane-1-sulfonic acid. The final concentrations of the compounds ranged from 500 to 0.975 μmol l^{-1}. Drug-free controls were included. The minimal inhibitory concentrations (MICs) were determined after 24 and 48 h of static incubation at 35 °C. With *T. mentagrophytes*, the final MICs were determined after 72 and 120 h of incubation. The results of all compounds in vitro tested against *T. mentagrophytes*, the most susceptible fungal strain, are summarized in Table 2.

4.15. Antimycobacterial assay

Antimycobacterial evaluation was carried out at the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), Southern Research Institute, Birmingham, AL, USA, which is a part of the National Institutes of Health (NIH). Primary screening of all compounds was conducted at 6.25 μg ml^{-1} against *M. tuberculosis* strain H37Rv in BACTEC 12B medium using the BACTEC 460 radiometric system [24,25]. The results are presented in Table 2.

Acknowledgements

Author is highly thankful to IOCL, R&D Center, Faridabad, Haryana, India for financial and technical support.

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