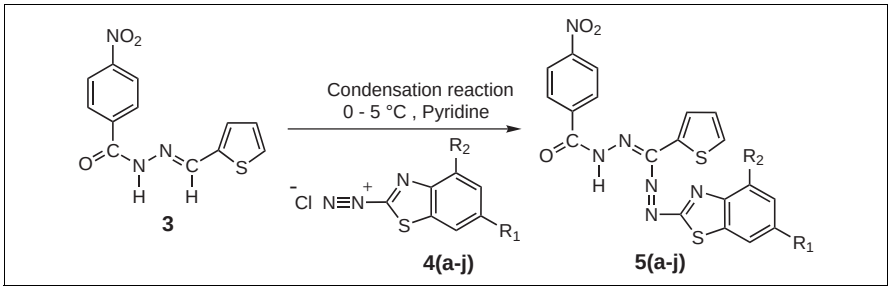
Krunal G. Desai[1]* and Kishor R. Desai[1]

[1]Department of Chemistry, Synthetic Organic Chemistry Research Laboratory,
Veer Narmad South Gujarat University, Udhna-magdalla Road,
Surat (West) - 395 007 (Gujarat state), India.
Ph: - +91-0261-2256012, Fax: - +91-0261-2256012
E-mail:- kgdapril@yahoo.co.in
Received September 30, 2005

Φ This paper is dedicated to Prof. K. R. Desai in recognition of his outstanding contributions to "Organic Chemistry".



Formazans **5(a-j)** have been prepared by the condensation between schiff base **3** and diazonium salt of substituted 2-amino benzothiazole **4(a-j)**. The intermediate schiff base **3** was synthesized by the condensation of *p*-nitrobenzolyhydrazide **1** with thiophene-2-aldehyde **2**. All the compounds have been screened for their antifungal activity against *Candida albicans (ATCC*-64550), *Candida krusei (ATCC-14243)* and *Candida parapsilosis (ATCC-22019)* and antibacterial activity against *Escherchia coli (ATCC-6538), Staphylococcus aureus (ATCC-6538), Pseudomonas aeruginosa (ATCC-1539)* and *Bacillus substilis (ATCC-6633).* The structures of the synthesised compounds **5(a-j)** have been characterized on the basis of their elemental analysis and spectral data (UV, IR, ^{1}H NMR, ^{13}C NMR and Mass).

J. Heterocyclic Chem., **43**, 1083 (2006).

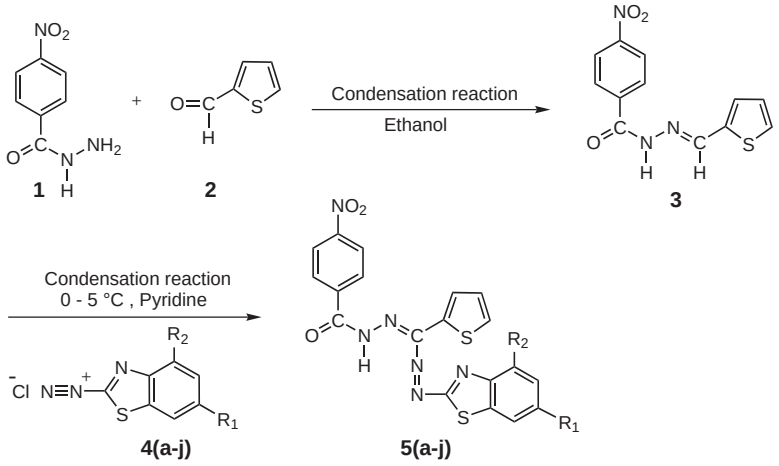
Introduction.

Schiffbase exhibit good antimicrobial activity [1] and pharmacological applications [1]. These compounds show good fungicidal activity [2] and their formazan derivatives possess antiviral [3], antimicrobial [4] and anti-inflammatory [5] activities. Number of formazans have been claimed to possess promising antifertility [6] and antiviral [6] activities particularly against Ranikhet disease virus and plant virus. Recently, jolly *et. al.* have synthesized new formazan for assessing their antiviral [7], anticancer [8] and anti HIV activities [9,10] and Desai *et. al.* have synthesized new formazan for assessing their antibacterial and antifungal properties [11]. Formazans are also used as antiparkinsonian agents [12]. Benzothiazole derivatives exhibit various biological activities [13]. Application of formazans in testing sensitivity of anticancer drugs has been mentioned by Bhardwaj [14]. Formazans are also used in the brucellaring test of milk [15] and the determination of the effects of anticancer drugs. All the compounds synthesised were adequately characterized by their elemental analysis and spectral data (UV, IR, ^{1}H NMR, ^{13}C NMR and Mass data).

Results and Discussion.

p-Nitrobenzolyhydrazide **1** on condensation reaction with thiophene-2-aldehyde **2** in ethanol as a reaction mediator afforded schiff base **3**. Formation of schiff base **3** was confirmed by appearance of ir spectra band in the region 1626 cm^{-1} corresponding -N=CH- group, 1667 cm^{-1} corresponding >C=O group of amide, 3350 cm^{-1} corresponding -NH- group (secondary amine) of schiff base **3** and disappearance of ir spectra band in the region 3378 cm^{-1} and 1710 cm^{-1} corresponding $-NH_2$ group and –CHO group of *p*-nitrobenzolyhydrazide **1** and thiophene-2-aldehyde **2** respectively. The ^{1}H nmr spectra showed a singlet at δ 4.45 ppm due to >N=CH- (1H) of schiff base **3**. The disappearance of signal at δ 2.60 ppm due to $-NH_2$ (2H) of *p*-nitrobenzolyhydrazide **1** and δ 9.5 ppm due to –CHO (1H) of thiophene-2-aldehyde **2** in ^{1}H nmr spectra. The ^{13}C nmr spectra showed a signal at δ 52.99 ppm due to >CH-N< of schiff base **3**. Further formation of these derivatives was confirmed by ms spectra, the molecular ion peak was observed at 275 m/z and base peak was observed at M^+- 18 by the loss of $-H_2O$ from the molecular ion.

Scheme 1



Schiff base **3** and diazonium salt of substituted 2-amino benzothiazole **4(a-j)** in pyridine at 0 – 5 ºC temperature as a reaction afforded substituted formazans **5(a-j)**. These compound show ir absorption band at 1625 cm^{-1} due to –C=N- group, 1665 cm^{-1} due to >C=O group of amide, 3352 cm^{-1} due to -NH- group (secondary amine) and 1570 cm^{-1} due to -N=N- group and the disappearance of bands at 1626 cm^{-1} (-N=CH-) also confirmed the formation of **5(a-j)**. In ^{1}H nmr spectra of formazans **5(a-j)** shows disappearance of singlet δ 4.45 ppm due to >C=NH– of compound **3**. In the ms spectra of formazan **5a** the molecular ion peak 481[M^+] also confirmed the formation of formazans. Fragment ion (m^+) peak was observed at 331 m/z ($C_{12}H_7O_2N_6S_2^+$), 316 m/z ($C_{12}H_6O_2N_5S_2^+$), 207 m/z ($C_7H_4O_2N_5S^+$), 179 m/z ($C_7H_4O_2N_3S^+$), 150 m/z ($C_7H_4NO_3^+$), 135 m/z ($C_6H_4O_2N_3^+$), 122 m/z ($C_6H_4NO_2^+$), 121 m/z ($C_6H_4O_2N_2^+$), 109 m/z ($C_5H_3NS^+$), 83 m/z ($C_4H_3S^+$) and 38 m/z ($C_3H_2^+$) by the loss of fragment radicals and neutrals •CO (-28), •NH (-15), •CN (-26), •CHS (-45), N_2 (-28), •CNS (-58) and •CS (-44). MS spectral fragmentation pattern is presented (Figure 1) as an additional evidence for the proposed structure **5a**. The synthetic route of above mentioned compounds is shown in Scheme 1.

Experiments were performed to evaluate the anti-bacterial activity against *Escherchia coli (ATCC-6538)*, *Staphylococcus aureus (ATCC-6538)*, *Pseudomonas aeruginosa (ATCC-1539)* and *Bacillus substilis (ATCC-6633)* using disk diffusion and the antifungal against *Candida albicans (ATCC-64550)*, *Candida krusei (ATCC-14243)* and *Candida parapsilosis (ATCC-22019)* using microdilution.

Antibacterial Activity.

The synthesized derivatives **5(a-j)** were screened for their *in vitro* antibacterial activity against *Escherchia coli (ATCC-6538)*, *Staphylococcus aureus (ATCC-6538)*, *Pseudomonas aeruginosa (ATCC-1539)* and *Bacillus substilis (ATCC-6633)* using disk diffusion method [16]. Mueller-Hinton agar (Difco, Detroit, USA) was used for bacterial strains. Ampicillin, amoxicillin and penicillin were also screened under similar conditions for comparison.

The results reveal the **5a**, **5e**, **5f**, **5g**, **5h** and **5i** exhibited the highest degree of inhibition against all tested organisms, while compounds **5a** and **5g** showed highest degree of inhibition only against *Escherchia coli (ATCC-6538)* species. Further, the data showed that the compounds **5a**, **5b**, **5d**, **5h** and **5j** are active for all species. Compounds **5c**, **5f**, **5g** and **5i** exhibited activity only against the *Staphylococcus aureus (ATCC-6538)* species. Compounds **5e**, **5f**, **5g** and **5i** exhibited activity only against the *Bacillus substilis (ATCC-6633)* species. Compounds **5c**, **5e**, **5f** and **5i** exhibited activity only against the *Pseudomonas aeruginosa (ATCC-1539)* species. However, the activities of the tested compounds are less than that of standard antibacterial agent used.

Antifungal Activity

Study design: Microdilution is used according to a standard protocol described by the NCCLS [17,18]. Three stains were tested each of the following species: *Candida albicans (ATCC-64550)*, *Candida krusei (ATCC-14243)* and *Candida parapsilosis (ATCC-22019)*.

Medium: RPMI 1640 broth with L-glutamine without sodium bicarbonate and 0.165 μ MOPS buffer (34.54 *g*/L) was used. The medium was adjusted to pH 7.0 at 25 ºC. Sterility of each bottle was performed before it was used.

Antifungal agents: Terbinafine was provided by the manufacturer as a standard powder. All drugs dissolved 100% dimethyl sulfoxide according to the NCCLS

Table I

MIC Values (μg/ml) of Compound **5(a-j)**

Compound	Antibacterial in (μg/ml)				Antifungal in (μg/ml)		
	Gram positive (+)		Gram negative (-)		*C. a* [e]	*C. k* [f]	*C. p* [g]
	S. a [a] *(ATCC 6538)*	*B. s* [b] *(ATCC 6633)*	*E. c* [c] *(ATCC 6538)*	*P. a* [d] *(ATCC 1539)*	*(ATCC 64550)*	*(ATCC 14243)*	*(ATCC 22019)*
5a	+	+ +	+ + +	+ +	-	+ + +	-
5b	+	+	+	+	+ + +	+	+ + +
5c	+ +	-	+ +	+ +	+ + +	+	+ +
5d	+	+	+ +	+ +	-	+ +	+
5e	-	+ + +	+ +	+ + +	+ + +	-	+ +
5f	+ +	+ + +	-	+ + +	+ +	+ + +	+ +
5g	+ +	+ +	+ + +	-	+	+ + +	-
5h	+ +	+ + +	+ +	+ +	+ + +	+	+ + +
5i	-	+ +	-	+ + +	+	+ + +	+
5j	+	+	+ +	+	+	+	-
	Zone of Inhibition of Standard Drugs (μg/ml)						
Ampicillin	+ + + +	+ + + +	+ + + + +	+ + + +	-	-	-
Amoxicillin	+ + + + +	+ + +	+ + + +	+ + + + +	-	-	-
Penicillin	+ + +	+ + + +	+ + + + +	+ + +	-	-	-
Flucanozole	-	-	-	-	+ + + + +	+ + + +	+ + + +

The inhibition diameter in mm: (-) < 6, (+) 7-10, (+ +) 11-16, (+ + +) 17-23, (+ + + +) 24-29, (+ + + + +) 30-36.

[a] *S. a - Staphylococcus aureus*; [b] *B. s - Bacills substilis*; [c] *E. c - Escherchia coli*; [d] *P. a – Pseudomonas aeruginosa*; [e] *C. a - Candida albicans*; [f] *C. k - Candida krusei*; [g] *Candida parapsilosis.*

Table II

Elemental Analysis and Physical Data of Compounds **5(a-j)**

Compound	Mol. formula (Mol. weight)	R_1	R_2	m. p/ºC	Yield (%)[a]	Anal. Calcd. (found)/ % C	H	N
5a	$C_{19}H_{11}O_5N_7S_2$ (481)	NO_2	H	196 ~ 197	68	47.40 (47.80	2.28 2.08	20.37 20.15)
5b	$C_{19}H_{11}O_3N_6S_2Cl$ (470.5)	Cl	H	158 ~ 159	73	48.45 (48.71	2.33 2.68	17.85 17.50)
5c	$C_{20}H_{14}O_3N_6S_2$ (450)	CH_3	H	198 ~ 199	66	53.33 (53.70	3.11 3.30	18.66 18.35)
5d	$C_{19}H_{12}O_3N_6S_2$ (436)	H	H	142 ~ 143	76	52.29 (52.58	2.75 2.35	19.26 19.40)
5e	$C_{19}H_{12}O_6N_6S_3$ (516)	SO_3H	H	275 ~ 278	75	44.18 (43.78	2.32 2.60	16.27 16.44)
5f	$C_{22}H_{18}O_3N_6S_2$ (478)	$CH(CH_3)_2$	H	237 ~ 238	59	55.23 (54.90	3.13 3.35	17.57 17.80)
5g	$C_{21}H_{15}O_4N_7S_2$ (493)	$NHCOCH_3$	H	246 ~ 247	63	51.11 (51.39	3.04 2.69	19.87 20.22)
5h	$C_{20}H_{14}O_4N_6S_2$ (466)	OCH_3	H	202 ~ 203	72	51.50 (51.80	3.00 2.71	18.02 18.42)
5i	$C_{19}H_{12}O_4N_6S_2$ (452)	OH	H	222 ~ 223	74	50.44 (50.70	2.65 2.95	18.58 18.25)
5j	$C_{19}H_{10}O_7N_8S_2$ (526)	NO_2	NO_2	201 ~ 202	70	43.34 (43.00	1.90 2.20	21.29 21.40)

[a]Yields refer to the isolated products.

methods [17,18]. The final drugs concentrations were 32 to 0.01 μg/mL for all drugs.

Preparation of inoculum: The preparation of inoculum suspensions was based mainly on the NCCLS guideline [18] and as described previously [19-21]. For dermatophytes the final inoculum size was adjusted from 1.2×10^4 to 6×10^4 CFU/mL and for *C. albicans* it was approximately 1×10^3 and 5×10^3 CFU/mL [17,22,23].

Test procedure: The test procedure was applied according to the NCCLS protocols [17,18]. Microdilution plates (96 U-shaped) were prepared and frozen at -70 ºC until needed. Each microdilution well containing 100 μL

Table III

UV, IR and MS Data of Compounds **5**(**a-j**)

Compound	uv (DMF) λ_{max} in nm	ir (KBr) ν_{max} in cm^{-1}	ms [K] (m/z)
5a	310	1661 (>C=O of amide, C=O str.), 3342, 1339 (>NH, N-H str., C-N str.), 1625 (-N=C<, C=N str. in schiff base), 1570 (-N=N- str. in formazan), 1615 (C=N str. in benzothiazole), 720 (C-S-C str.), 3023, 1510 (aromatic ring, C-H str., C=C str.), 1518, 1342 (-NO_2, N=O str.)	481 [M^+], 331 ($C_{12}H_7O_2N_6S_2^+$), 316 ($C_{12}H_6O_2N_5S_2^+$), 207 ($C_7H_4O_2N_5S^+$), 179 ($C_7H_4O_2N_3S^+$), 150 ($C_7H_4NO_3^+$), 135 ($C_6H_4O_2N_3^+$), 122 ($C_6H_4NO_2^+$), 121 ($C_6H_4O_2N_2^+$), 109 ($C_5H_3NS^+$), 83 ($C_4H_3S^+$), 38 ($C_3H_2^+$).
5b	295	1665 (>C=O of amide, C=O str.), 3339, 1342 (>NH, N-H str., C-N str.), 1621 (-N=C<, C=N str. in schiff base), 1573 (-N=N- str. in formazan), 1617 (C=N str. in benzothiazole), 718 (C-S-C str.), 3020, 1513 (aromatic ring, C-H str., C=C str.), 1512, 1344 (-NO_2, N=O str.), 835 (-Cl, C-Cl str.)	470 [M^+], 320 ($C_{12}H_7N_5S_2Cl^+$), 305 ($C_{12}H_6N_4S_2Cl^+$), 196 ($C_7H_3N_3SCl^+$), 168 ($C_7H_3NSCl^+$), 150 ($C_7H_4NO_3^+$), 124 ($C_6H_3NCl^+$), 122 ($C_6H_4NO_2^+$), 110 ($C_6H_3Cl^+$), 109 ($C_5H_3NS^+$), 83 ($C_4H_3S^+$), 38 ($C_3H_2^+$).
5c	291	1667 (>C=O of amide, C=O str.), 3341, 1345 (>NH, N-H str., C-N str.), 1623 (-N=C<, C=N str. in schiff base), 1576 (-N=N- str. in formazan), 1620 (C=N str. in benzothiazole), 720 (C-S-C str.), 3021, 1515 (aromatic ring, C-H str., C=C str.), 1513, 1346 (-NO_2, N=O str.), 1310 (-CH_3, C-H bend.)	450 [M^+], 300 ($C_{13}H_{10}N_5S_2^+$), 285 ($C_{13}H_9N_4S_2^+$), 176 ($C_8H_6N_3S^+$), 150 ($C_7H_4NO_3^+$), 148 ($C_8H_6NS^+$), 122 ($C_6H_4NO_2^+$), 109 ($C_5H_3NS^+$), 104 ($C_7H_6N^+$), 90 ($C_7H_6^+$), 83 ($C_4H_3S^+$), 38 ($C_3H_2^+$).
5d	324	1664 (>C=O of amide, C=O str.), 3340, 1342 (>NH, N-H str., C-N str.), 1620 (-N=C<, C=N str. in schiff base), 1572 (-N=N- str. in formazan), 1617 (C=N str. in benzothiazole), 716 (C-S-C str.), 3018, 1512 (aromatic ring, C-H str., C=C str.), 1510, 1343 (-NO_2, N=O str.)	436 [M^+], 286 ($C_{12}H_8N_5S_2^+$), 271 ($C_{12}H_7N_4S_2^+$), 162 ($C_7H_4N_3S^+$), 150 ($C_7H_4NO_3^+$), 134 ($C_7H_4NS^+$), 122 ($C_6H_4NO_2^+$), 109 ($C_5H_3NS^+$), 90 ($C_6H_4N^+$), 76 ($C_6H_4^+$), 83 ($C_4H_3S^+$), 38 ($C_3H_2^+$).
5e	297	1666 (>C=O of amide, C=O str.), 3343, 1347 (>NH, N-H str., C-N str.), 1624 (-N=C<, C=N str. in schiff base), 1577 (-N=N- str. in formazan), 1622 (C=N str. in benzothiazole), 722 (C-S-C str.), 3023, 1513 (aromatic ring, C-H str., C=C str.), 1516, 1349 (-NO_2, N=O str.), 1220, 1040 (-SO_3H, S=O str.)	516 [M^+], 366 ($C_{12}H_8O_3N_5S_3^+$), 351 ($C_{12}H_7O_3N_4S_3^+$), 342 ($C_7H_4O_3N_3S_2^+$), 314 ($C_7H_4O_3N_2S_2^+$), 270 ($C_6H_4O_3N_2S^+$), 256 ($C_6H_4O_3NS^+$), 150 ($C_7H_4NO_3^+$), 122 ($C_6H_4NO_2^+$), 109 ($C_5H_3NS^+$), 83 ($C_4H_3S^+$), 38 ($C_3H_2^+$).
5f	278	1662 (>C=O of amide, C=O str.), 3341, 1343 (>NH, N-H str., C-N str.), 1622 (-N=C<, C=N str. in schiff base), 1573 (-N=N- str. in formazan), 1620 (C=N str. in benzothiazole), 721 (C-S-C str.), 3022, 1511 (aromatic ring, C-H str., C=C str.), 1513, 1350 (-NO_2, N=O str.), 1362 (-$CH(CH_3)_2$, C-H bend.)	478 [M^+], 328 ($C_{15}H_{14}N_5S_2^+$), 313 ($C_{15}H_{13}N_4S_2^+$), 204 ($C_{10}H_{10}N_3S^+$), 176 ($C_{10}H_{10}NS^+$), 150 ($C_7H_4NO_3^+$), 132 ($C_9H_{10}N^+$), 122 ($C_6H_4NO_2^+$), 118 ($C_9H_{10}^+$), 109 ($C_5H_3NS^+$), 83 ($C_4H_3S^+$), 38 ($C_3H_2^+$).
5g	272	1659 (>C=O of amide, C=O str.), 3339, 1340 (>NH, N-H str., C-N str.), 1619 (-N=C<, C=N str. in schiff base), 1569 (-N=N- str. in formazan), 1622 (C=N str. in benzothiazole), 723 (C-S-C str.), 3023, 1516 (aromatic ring, C-H str., C=C str.), 1517, 1351 (-NO_2, N=O str.), 1582 (-$NHCOCH_3$, N-H bend.)	493 [M^+], 343 ($C_{14}H_{11}ON_6S_2^+$), 328 ($C_{14}H_{10}ON_5S_2^+$), 219 ($C_9H_7ON_4S^+$), 191 ($C_9H_7ON_2S^+$), 150 ($C_7H_4NO_3^+$), 147 ($C_8H_7ON_2^+$), 133 ($C_8H_7ON^+$), 122 ($C_6H_4NO_2^+$), 109 ($C_5H_3NS^+$), 83 ($C_4H_3S^+$), 38 ($C_3H_2^+$).
5h	332	1662 (>C=O of amide, C=O str.), 3344, 1348 (>NH, N-H str., C-N str.), 1623 (-N=C<, C=N str. in schiff base), 1562 (-N=N- str. in formazan), 1628 (C=N str. in benzothiazole), 728 (C-S-C str.), 3028, 1518 (aromatic ring, C-H str., C=C str.), 1517, 1356 (-NO_2, N=O str.), 2825 (-OCH_3, C-H str.)	466 [M^+], 316 ($C_{13}H_{10}ON_5S_2^+$), 301 ($C_{13}H_9ON_4S_2^+$), 192 ($C_8H_6ON_3S^+$), 164 ($C_8H_6ONS^+$), 150 ($C_7H_4NO_3^+$), 122 ($C_6H_4NO_2^+$), 120 ($C_7H_6ON^+$), 109 ($C_5H_3NS^+$), 106 ($C_7H_6O^+$), 83 ($C_4H_3S^+$), 38 ($C_3H_2^+$).
5i	301	1667 (>C=O of amide, C=O str.), 3349, 1341 (>NH, N-H str., C-N str.), 1625 (-N=C<, C=N str. in schiff base), 1568 (-N=N- str. in formazan), 1621 (C=N str. in benzothiazole), 724 (C-S-C str.), 3030, 1520 (aromatic ring, C-H str., C=C str.), 1519, 1352 (-NO_2, N=O str.), 3590 (-OH, O-H str.)	452 [M^+], 302 ($C_{12}H_8ON_5S_2^+$), 287 ($C_{12}H_7ON_4S_2^+$), 178 ($C_7H_4ON_3S^+$), 150 ($C_7H_4NO_3^+$), 122 ($C_6H_4NO_2^+$), 109 ($C_5H_3NS^+$), 106 ($C_6H_4ON_3^+$), 92 ($C_6H_4ON_2^+$), 83 ($C_4H_3S^+$), 38 ($C_3H_2^+$).
5j	285	1661 (>C=O of amide, C=O str.), 3342, 1344 (>NH, N-H str., C-N str.), 1622 (-N=C<, C=N str. in schiff base), 1568 (-N=N- str. in formazan), 1623 (C=N str. in benzothiazole), 726 (C-S-C str.), 3035, 1522 (aromatic ring, C-H str., C=C str.), 1511, 1351 (-NO_2, N=O str.)	526 [M^+], 376 ($C_{12}H_6O_4N_7S_2^+$), 361 ($C_{12}H_5O_4N_6S_2^+$), 252 ($C_7H_2O_4N_5S^+$), 224 ($C_7H_2O_4N_3S^+$), 180 ($C_6H_2O_4N_3^+$), 166 ($C_6H_2O_4N_2^+$), 150 ($C_7H_4NO_3^+$), 122 ($C_6H_4NO_2^+$), 109 ($C_5H_3NS^+$), 83 ($C_4H_3S^+$), 38 ($C_3H_2^+$).

[K] Source temperature - 250° C; Sample temperature - 190° C; Reservoir – 75 eV.

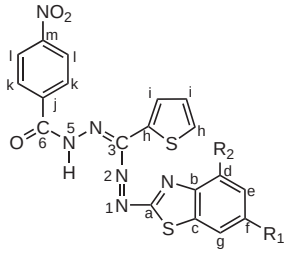
of the 2-fold drug concentration was inoculated 100 μL of the final inoculum suspension. Two drug-free growth controls were included for each test plate, one without any drug (growth control) and the other with media containing an equivalent amount of solvent used to dissolved the drug (solvent control). For all drugs, the minimum inhibitory concentration (MIC) was defined as the lowest concentration showing 100% growth inhibition. All of the compounds **5(a-j)** were found to have antifungal activity against *Candida albicans (ATCC-64550)*, *Candida krusei (ATCC-14243)* and *Candida parapsilosis (ATCC-22019)*. MIC values of the compounds are given in Table I.

Table IV

^{1}H nmr and ^{13}C nmr data of compounds **5(a-j)**

Compound	^{1}H nmr [G] ($CDCl_3$ - DMSO-d_6) δ in ppm	^{13}C nmr [G] ($CDCl_3$ – DMSO-d_6) δ in ppm
5a	6.70 - 7.90 (m, 10H, Ar-H), 11.52 (s, 1H, -CONH-)	165.81 (C-6), 52.99 (C-3), 153.90 (C-a), 153.15 (C-b), 133.61 (C-c), 126.11 (C-d), 125.45 (C-e), 140.65 (C-f), 121.80 (C-g), 125.14 (C-h), 126.92 (C-i), 134.78 (C-j), 129.49 (C-k), 123.51 (C-l), 148.37 (C-m)
5b	7.09 – 8.30 (m, 10H, Ar-H), 11.57 (s, 1H, -CONH-)	164.88 (C-6), 53.11 (C-3), 154.80 (C-a), 152.20 (C-b), 132.66 (C-c), 127.02 (C-d), 126.38 (C-e), 134.33 (C-f), 122.76 (C-g), 124.08 (C-h), 125.82 (C-i), 135.71 (C-j), 130.43 (C-k), 124.46 (C-l), 149.30 (C-m)
5c	7.10 - 8.32 (m, 10H, Ar-H), 11.56 (s, 1H, -CONH-), 2.34 (s, 3H, $-CH_3$)	165.83 (C-6), 53.18 (C-3), 153.99 (C-a), 153.24 (C-b), 133.69 (C-c), 126.10 (C-d), 125.33 (C-e), 137.83 (C-f), 121.80 (C-g), 125.06 (C-h), 126.81 (C-i), 134.78 (C-j), 129.45 (C-k), 123.41 (C-l), 148.34 (C-m), 21.41 ($-CH_3$)
5d	6.91 - 8.42 (m, 11H, Ar-H), 11.62 (s, 1H, -CONH-)	165.88 (C-6), 53.11 (C-3), 153.80 (C-a), 153.20 (C-b), 133.66 (C-c), 126.02 (C-d), 125.38 (C-e), 123.52 (C-f), 121.76 (C-g), 125.08 (C-h), 126.82 (C-i), 134.71 (C-j), 129.43 (C-k), 123.46 (C-l), 148.30 (C-m)
5e	7.11 - 8.47 (m, 10H, Ar-H), 11.64 (s, 1H, -CONH-), 8.40 (s, 1H, $-SO_3H$)	166.87 (C-6), 54.15 (C-3), 154.80 (C-a), 153.29 (C-b), 133.89 (C-c), 125.99 (C-d), 125.88 (C-e), 124.00 (C-f), 122.70 (C-g), 125.57 (C-h), 126.08 (C-i), 133.99 (C-j), 129.45 (C-k), 122.55 (C-l), 147.31 (C-m)
5f	7.10 - 8.37 (m, 10H, Ar-H), 11.77 (s, 1H, -CONH-), 2.87 (q, 1H, -CH-), 1.24 (d, 3H, $-CH_3$)	165.68 (C-6), 53.31 (C-3), 153.60 (C-a), 153.40 (C-b), 133.86 (C-c), 126.13 (C-d), 125.56 (C-e), 148.83 (C-f), 121.78 (C-g), 124.08 (C-h), 126.62 (C-i), 134.91 (C-j), 129.53 (C-k), 123.66 (C-l), 148.33 (C-m), 34.18 (-CH-), 24.02 ($-CH_3$)
5g	7.08 - 8.39 (m, 10H, Ar-H), 11.69 (s, 1H, -CONH-), 7.79 (s, 1H, -NH-), 2.138 (s, 3H, $-CH_3$)	166.86 (C-6), 54.14 (C-3), 154.81 (C-a), 153.30 (C-b), 133.88 (C-c), 125.97 (C-d), 125.89 (C-e), 138.15 (C-f), 122.72 (C-g), 125.53 (C-h), 126.08 (C-i), 132.99 (C-j), 129.47 (C-k), 123.55 (C-l), 147.34 (C-m), 169.48 (-CO-), 24.18 ($-CH_3$)
5h	6.99 - 8.37 (m, 10H, Ar-H), 11.67 (s, 1H, -CONH-), 3.95 (s, 3H, $-OCH_3$)	165.81 (C-6), 53.15 (C-3), 153.83 (C-a), 153.25 (C-b), 133.61 (C-c), 126.05 (C-d), 125.40 (C-e), 123.51 (C-f), 121.79 (C-g), 125.08 (C-h), 126.87 (C-i), 134.75 (C-j), 129.41 (C-k), 122.46 (C-l), 147.30 (C-m), 35.7 ($-OCH_3$)
5i	7.32 - 8.31 (m, 10H, Ar-H), 11.60 (s, 1H, -CONH-), 3.65 (s, 1H, -OH)	166.87 (C-6), 54.15 (C-3), 154.80 (C-a), 153.29 (C-b), 133.89 (C-c), 125.99 (C-d), 125.88 (C-e), 124.00 (C-f), 122.70 (C-g), 125.57 (C-h), 126.08 (C-i), 133.99 (C-j), 129.45 (C-k), 122.55 (C-l), 147.31 (C-m)
5j	7.21 - 8.32 (m, 9H, Ar-H), 11.78 (s, 1H, -CONH-)	165.81 (C-6), 52.99 (C-3), 153.90 (C-a), 153.15 (C-b), 133.61 (C-c), 126.11 (C-d), 125.45 (C-e), 140.65 (C-f), 121.80 (C-g), 125.14 (C-h), 126.92 (C-i), 134.78 (C-j), 129.49 (C-k), 123.51 (C-l), 148.37 (C-m)

[G] 400 MHz; Sample – 0.038g=0.5 mL DMSO-d_6; 17.5 mg=0.5 mL $CDCl_3$.



5(a-j)

The results reveal that **5b**, **5c**, **5e**, **5f**, **5h** and **5i** exhibited the highest degree of inhibition against all tested organisms, while compounds **5b**, **5c**, **5e** and **5h** showed highest degree of inhibition only against *Candida albicans (ATCC-64550)* species. Further, the data showed that the compounds **5b**, **5c**, **5f** and **5h** are active for all species. Compounds **5a**, **5f**, **5g** and **5i** exhibited activity only against the *Candida krusei (ATCC-14243)* species. Compounds **5b** and **5g** exhibited activity only against the *Candida parapsilosis (ATCC-22019)* species. Flucanozole was also screened under similar conditions for comparison. However, the activities of the tested compounds are less than that of standard antifungal agent used.

EXPERIMENTAL

General.

All the melting points were determined in PMP-DM scientific melting point apparatus and are uncorrected. The purity of compounds was checked routinely by TLC (0.5 mm thickness) using silica gel-G coated Al plates (Merck) and spots were visualized by exposing the dry plates in iodine vapours. The IR spectra (ν_{max} in cm^{-1}) were recorded on a shimadzu FT-IR 8300 spectrophotometer using KBr or Nujol technique; UV spectra (λ_{max} in nm) were recorded on a shimadzu UV – 160 A (200-400 nm) on using DMF as solvent; ^{1}H NMR spectra on a Bruker WM 400FT MHz NMR instrument using $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal reference (chemical shifts in δ, ppm); ^{13}C NMR on a Varian AMX 400 (400 MHz) spectrometer as solutions in $CDCl_3$ (chemical shifts in δ, ppm) and Mass spectra on a Jeol JMS D-300 spectrometer. The elemental analysis (C, H, N) of compounds was performed on Carlo Erba-1108 elemental analyzer.

Synthesis of *p*-nitrobenzoylhydrazide (**1**).

This compound was synthesized according to the method of Desai *et. al.* [11].

Synthesis of N'-thiophelidene-N-benzoylhydrazides (**3**).

A solution of compound 1 (0.01 mole, 1.81 g) and thiophene-2-aldehyde **2** (0.01 mole, 1.12 mL) in ethanol (30 mL) was refluxed for 5 - 6 hr. The solvent was removed by vacuum distillation. The solid product **3** obtained was collected by filtration, dried and recrystallised from absolute ethanol. Yield 2.11 g (76.72 %); mp 166 ˚C; ir (ν_{max} in cm^{-1}): 1664 (>C=O of amide, C=O str.), 3345, 1335 (>NH, N-H str., C-N str.), 1625 (-N=C<, C=N str. in schiff base), 718 (C-S-C str.), 3021, 1515 (aromatic ring, C-H str., C=C str.), 1516, 1340 (-NO_2, N=O str.); ^{1}H nmr (δ in ppm): 6.78 - 7.89 (m, 7H, Ar-H), 11.48 (s, 1H, -CONH-), 4.45 (s, 1H, -N=CH-); ms (m/z): 275 [M^+], 150 ($C_7H_4NO_3^+$), 125 ($C_5H_5N_2S^+$), 122 ($C_6H_4NO_2^+$), 110 ($C_5H_4NS^+$), 83 ($C_4H_3S^+$), 38 ($C_3H_2^+$).

Anal. Calcd. For $C_{12}H_9O_3N_3S$: C, 52.36; H, 3.27; N, 15.27. Found: C, 51.98; H, 3.47; N, 14.92 %.

General Procedure of 1-(Substitutedbenzothiazole)-3-(thiophel-idene)-5-(4'-nitrobenzoyl) formazans (**5a-j**).

Substituted 2-amino benzothiazole [24] (0.01 mole) was dissolved in aq. HCl (10 mL). It was cooled and aq. $NaNO_2$ (0.7 g) was slowly added. N'-thiophelidene-N-benzoylhydrazide **3** (0.01 mole, 2.75 g) was dissolved in dry pyridine (10 mL) and sodium acetate (0.3 g) was added. The contents were cooled in an ice-bath and stirred. To it a clear and cold solution of diazonium salt of substituted 2-amino benzothiazole **4(a-j)** was added dropwise for 1 hr at low temperature (0-5˚C). The reaction mixture was kept in ice-bath for 3 hr and then poured into ice water. The resulting dark coloured mass was collected by filtration, washed with water till it was free from pyridine and dried. The product was crystallised from ethanol. The analytical data and spectral data for different substituted formazans **5**(**a-j**) are given in Table II, Table III and Table IV.

Acknowledgement.

The Authors are thankful to the Head, Department of Chemistry, Veer Narmad South Gujarat University, Surat for providing research facilities and one of the author (Krunal Desai) is also thankful to Gujarat Council On Science and Technology (GUJCOST), Gandhinagar (Grant no. GUJCOST/ 200389/MRP/2003-04/10689) for financial assistance. The authors are also thankful to the Central Drug Research Institute (CDRI), Lucknow for providing spectral and analytical data of the compounds and thankful to the Head of Bioscience Department of VNSGU, Surat for biological screening.

REFERENCES AND NOTES

[1] K. G. Desai and K. R. Desai, *Indian J. Chem.* **44B**, 2097 (2005).
[2] J. Slouka, T. Gucky and I. Frysova, *Arkivoc (USA), (www.arkat-usa.org).*, **XV**, 3 (2005).
[3] V. K. Pandey and H. S. Negi, *Indian Drugs (Indian Drug Manufactures Association)*, **36(1)**, 37 (1999).
[4] R. M. Desai and J. M. Desai, *Indian J. Heterocycl. Chem.*, **8(4)**, 329 (1999).
[5] E. Stefanescu, M. Dorneanu, C. Danila, C. Aprotosoaie, G. Grosu and M. Pavelescu, *Rev. Med. Chir. Soc. Med. Nat. Iasi.*, **101(3-4)**, 182 (1997).
[6] J. M. Desai and V. H. Shah, *Indian J. Chem.*, **42B**, 631 (2003).
[7] T. Hongo and Y. Fuji, *She Med Hammanutsec Univ Hammamtry Japan*, **65**, 1263 (1990).
[8] S. D. Bhardwaj and V. S. Jolly, *Int. J. Chem. Sci.*, **1(3)**, 276 (2003).
[9] N. K. Venkal, *J. Med. Chem.* **8**, 11 (1998).
[10] S. D. Bhardwaj, V. S. Jolly, *Asian J. Chem.*, **14**, 767 (2002).
[11] K. G. Desai and K. R. Desai, *Indian J. Chem.*, **44B**, 2093 (2005).
[12] R. Khanna, A. K. Saksena, K. Sanker, *Indian J. Chem.*, **29B**, 94 (1990).
[13] K. M. Mistry and K. R. Desai, *Indian J. Chem.* **44B**, 1455 (2005).
[14] S. D. Bhardwaj, *Asian J. Chem.* **10(1)**, 39-42 (1998).
[15] S. D. Bhardwaj, P. Phatak and V. S. Jolly, *Orient. J. Chem.*, **2**, 181 (1995).
[16] National committee for clinical laboratory standards, (2000).
[17] National committee for clinical laboratory standards, (1997).
[18] National committee for clinical laboratory standards, (1998).
[19] B. Fernandez, A. J. Cabanes and I. Inza. *J. Clinical Microbial.*, **40**, 4003 (2002).
[20] B. Fernandez, A. J. Carillo and E. Martin, *Antimicrob. Agents Chemother.*, **45**, 2528 (2001).

[21] A. K. Gupta and Y. Kohli, *Brit. J. Dermatol*., **149**, 305 (2003).

[22] C. W. Kigh, R. A. Famthing and M. G. Rinaldi, *J. Clinical Microbial*., **30**, 3145 (1992).

[23] J. Berenguer, R. Sanchez and J. V. Matinez, *Antimicrob. Agents Chemother*., **40**, 2003 (1996).

[24] K. G. Ojha, H. Tahiliani and N. Jaisinghani, *Chemistry An Indian Journal*, **1**, 171 (2003).