SYNTHESIS, CHARACTERIZATION AND WOUND HEALING ACTIVITY OF TETRAZOLOQUINOLINE THIOCARBOHYDRAZIDE DERIVATIVES

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ABSTRACT
Ten novel Schiff’s bases of N″-[tetrazolo[1,5-a]quinoline-4 ylmethylidene] thiocarbohydrazide derivatives 6a-j were synthesized. All the compounds have been characterized by IR, 1HNMR and Mass spectroscopy. To validate the ethnotherapeutic claims of the synthetic compounds in skin diseases, wound healing activity of few selected synthesized compounds were studied in albino rats by excision wound model using povidine iodine as reference standard, these titled compounds exhibited significant wound healing activity.

KEYWORDS
Schiff’s bases, 2-Chloro-3-formyl-quinoline, Tetrazoloquinoline, Thioarbo hydrazide and Wound healing activity.

INTRODUCTION
Wounds are physical injuries that result in an opening or breaking of the skin. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. It is a product of the integrated response of several cell types to injury. Wound healing is a complex multi factorial process that they

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results in the contraction and closure of the wound and restoration of a functional barrier. Wound healing is a dynamic process involving biochemical and physiological phenomena that behave in a harmonious way in order to guarantee tissue restoration.

Quinolines have occupied a unique place in medicinal chemistry due to their diverse pharmacological displays such as anti-leishmanial, antibacterial, antimalarial, and antifungal activities. The tetrazole group, which is considered as an analogue to the carboxylic group as a pharmacore, possesses a wide range of biological activities. Several substituted tetrazoles have been shown to possess anti-inflammatory, antibacterial, anti-aids, anticancer, antifungal, and anticonvulsant activities. The development of tetrazole chemistry has been largely associated with wide-scale applications of these classes of compounds in medicine, biochemistry, agriculture, and also a large number of medicinally important tetrazole heterocyclic incorporated drugs approved by the FDA. The tetrazole functionality plays an important role in medicinal chemistry, primarily due to its ability to serve as bioisostere of the carboxylic acid group. The fusion of quinoline with tetrazole ring is known to increase the biological activity. Tetrazoloquinoline derivatives possess excellent biological activities like antimicrobial, anticancer and anti-convulsant activities.

The Schiff bases derived from thiocarbohydrazide are known to exhibit diverse activities such as antibacterial, anticarcinogenic, and antifungal activities. Furthermore, Schiff bases are utilized as starting materials in the synthesis of industrial important compounds. Inspired by the significance of tetrazoloquinolines, the Schiff’s bases of thiocarbohydrazide, in the present work, an attempt has been done to design and synthesize novel compounds which contain all these pharmacophores that is tetrazole, quinoline and imine functionality with thiocarbohydrazides.

MATERIAL AND METHOD
All chemicals (analytical grade) were purchased from S. D. Fine, Mumbai. Melting points of all the synthesized compounds were determined by open capillary tube method and are uncorrected. The purity of all compounds was determined by TLC plates precoated with Silica Gel-G (E. Merck, Mumbai) by using Chloroform and Ethanol (9.5:0.5) as solvent system. Spots were visualized in iodine vapour chamber. IR spectra were recorded on SHIMADZU FTIR-8400S spectrophotometer by using KBr pellets technique. HNMR was recorded on Bruker AMX 400 MHz spectrophotometer by using DMSO as solvent. Mass spectra were recorded on a Jeol JMS-D 300 mass spectrophotometer.

EXPERIMENTAL
2-Chloroquinoline-3-carbaldehyde [1] was synthesized by literature method. Synthesis of Tetrazolo-[1,5-a]quinoline-4-carbaldehyde [2] Into a solution of 2-Chloroquinoline-3-carbaldehyde (0.001 mol, 0.191 gm) in absolute ethanol (5 ml), p-toluenesulphonic acid (0.001 mol, 0.190 gm) and sodium azide (0.0015 mol, 0.0975 gm) were added and the reaction mixture was refluxed for 65 hours at 125-135 °C. After completion of the reaction (monitored by TLC), the reaction mixture was poured into ice cold water (100 ml) and the resulting precipitate was filtered, dried and recrystallized from dimethyl formamide as whitish light yellow needle shaped crystals. Yield 76 %, m.p. 240-242 °C.

Thiocarbohydrazide [3] was prepared literature method. Synthesis of N’’-[tetrazolo [1,5-a] quinoline-4-ylmethylidene] thiocarbohydrazide [4] In 250 ml round bottom flask the solution of tetrazolo[1,5-a]quinoline-4-carbaldehyde (0.01 mol, 1.98 gm) in 1,4-Dioxane (25 ml), was added an equivalent amount of thiocarbohydrazide (0.01 mol, 1.06 gm). The reaction mixture was refluxed for 8 hours at 155-160 °C, partially concentrated and
cooled. The separated solid product was filtered, dried and recrystalized from dimethyl formamide to give a light yellow powder. Yield 64.3 %, m.p. 224-226 °C.

**Synthesis of N″-[(tetrazolo[1,5-α]quinolin-4-ylmethylidene]thiocarbohydrazide derivatives (6a-j)

**General Procedure**
The solution of N″-[(tetrazolo[1,5-α]quinoline-4-ylmethylidene] thiocarbo -hydrazide (0.1 mol, 0.286 gm) in dimethyl formamide (50 ml) and the proper aromatic aldehyde (0.1 mol) were added in to a clean dry round-bottom flask. The reaction mixture was refluxed for 24-30 hours at 160-170 °C, then cooled further it was poured into ice cold water (100 ml). The separated solid was filtered, recrystalized from aqueous dimethyl formamide to give a pure light brown crystalline powder. Physical data of synthesized compounds is given in Table No.1.

**SCHEME-I**

![SCHEME-I](image-url)
Wound healing activity

Selected synthesized compounds were evaluated for their wound healing activity in albino rats by excision wound model taking povidone iodine as reference standard. Albino rats weighing 200 - 250 g were used. They were kept in a standard environmental condition and fed with rodent diet and water ad libitum. In the experiment, the rats were divided into six groups. Group one was the control group which received simple ointment base, group two was treated with reference standard (5% w/w povidone iodine) and groups three to six received our newly synthesized compounds 6(a,d,f and h) containing 2.5% w/w ointment topically on wound created on the dorsal back of rats daily till the wounds completely healed. Excision wound model Full thickness excision wound was made on the shaved back of the rat by removing a 400 sq mm piece of skin and the day on which wound was made was considered as day zero.

The various groups were treated as follows

Group-I: Control (0.5 gm, of simple ointment (vehicle) applied locally) Group-II: Standard (5 % w/w Povidone iodine ointment applied locally) Group-III: 6a, Group-IV: 6d, Group-V: 6f, Group-VI: 6h (0.5 gm, of 2.5% w/w ointment of test compounds applied locally once a day till complete epithelization.

Animals divided into six groups were treated as described above. The percent of wound closure was recorded on days 4, 8, 12 and 16 and the wound area was traced and measured planimetrically. The actual value was converted into percent value taking the size of the wound at the time of wounding as 100%. Results of wound healing activity are depicted in Table No.2.

The percentage wound closure was calculated using the following formula.

\[
\text{Percentage wound closure} = \left(1 - \frac{\text{Ad}}{\text{Ao}}\right) \times 100
\]

Where,
\[
\text{Ao} = \text{Wound area on day zero (400 sq. mm)}
\]

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RESULTS

Spectral data

6c: IR (KBr) cm\(^{-1}\): 2838 (C-H), 1619 (C=N), 1509 (C=C), 970 (N-N), 1252 (C=S), 3370 (N-H). \(^1\)H NMR (DMSO) δ ppm: 2.37 (s, 3H, CH\(_3\)), 7.320-8.134 (m, 9H, Ar-H), 9.295 (s, 1H, S=C-NH), 8.657 (s, 1H, -CH=N). ESIMS (m/z): 388 (M\(^+\)).

6d: IR (KBr) cm\(^{-1}\): 2849 (C-H), 1632 (C=N), 1508 (C=C), 963 (N-N), 1227 (C=S), 3295 (N-H). \(^1\)H NMR (DMSO) δ ppm: 7.228-8.368 (m, 9H, Ar-H), 9.127 (s, 1H, S=C-NH), 8.716 (s, 1H, -CH=N). ESIMS (m/z): 392 (M\(^+\)).

6f: IR (KBr) cm\(^{-1}\): 2848 (C-H), 1656 (C=N), 1493 (C=C), 958 (N-N), 1213 (C=S), 3370 (N-H). \(^1\)H NMR (DMSO) δ ppm: 6.406-7.884 (m, 10H, Ar-H), 9.075 (s, 1H, S=C-NH), 8.719 (s, 1H, -CH=N). ESIMS (m/z): 374 (M\(^+\)).

6h: IR (KBr) cm\(^{-1}\): 2850 (C-H), 1617 (C=N), 1528 (C=C), 960 (N-N), 1214 (C=S), 3271 (N-H). \(^1\)H NMR (DMSO) δ ppm: 7.251-8.362 (m, 9H, Ar-H), 8.889 (s, 1H, S=C-NH), 8.686 (s, 1H, -CH=N). ESIMS (m/z): 419 (M\(^+\)).

6j: IR (KBr) cm\(^{-1}\): 2837 (C-H), 1623 (C=N), 1509 (C=C), 962 (N-N), 1262 (C=S), 3372 (N-H). \(^1\)H NMR (DMSO) δ ppm: 3.827 (s, 6H, OCH\(_3\)), 7.063-8.414 (m, 8H, Ar-H), 9.147 (s, 1H, S=C-NH), 8.631 (s, 1H, -CH=N). ESIMS (m/z): 434 (M\(^+\)).

DISCUSSION

Wound healing activities of the some selected synthesized compounds 6-a, d, f & h were assessed against excision wound model in albino rats. For this purpose the compounds were formulated as 2.5% w/w ointment base.
% w/w ointment using simple ointment IP as vehicle. Povidine iodine 5 % w/w ointment used as reference standard drug.
The results of the present investigation indicate that all the four compounds on topical application in the form of ointment significantly promoted wound healing activity. The significant wound healing efficacy was evident by increase in rate of wound contraction and marked reduction in epithelization period.
Among the screened compounds for the wound healing study, the compound 6d has showed almost equipotent wound healing activity to that of reference standard drug Povidine iodine. The percentage wound closure of 6d and period of epithelization time (96.33 and 18.33) were found to be closer to that of standard Povidine iodine treated group (97.80 and 17.66). The Compound 6d also promoted wound healing property (94.90 and 18.50) which is nearer to standard.
The order of the wound healing efficacy of the test compound was found as 6d > 6a > 6h > 6f respectively.
From the results obtained, it was observed that the compound showed greater wound healing potential than the other compounds.

<p>| Table No.1: Characterization data of the newly synthesized compounds |
|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>S.No</th>
<th>Compound code</th>
<th>R</th>
<th>M. P.º C</th>
<th>Yield (%)</th>
<th>Rf</th>
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<tr>
<td>1</td>
<td>6a</td>
<td>4-OCH3</td>
<td>110</td>
<td>57</td>
<td>0.88</td>
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<tr>
<td>2</td>
<td>6b</td>
<td>2-OH</td>
<td>164</td>
<td>23</td>
<td>0.61</td>
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<td>3</td>
<td>6c</td>
<td>4-CH3</td>
<td>100</td>
<td>57</td>
<td>0.77</td>
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<tr>
<td>4</td>
<td>6d</td>
<td>4-Cl</td>
<td>160</td>
<td>54</td>
<td>0.83</td>
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<tr>
<td>5</td>
<td>6e</td>
<td>4-F</td>
<td>136</td>
<td>66</td>
<td>0.81</td>
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<tr>
<td>6</td>
<td>6f</td>
<td>H</td>
<td>84</td>
<td>67</td>
<td>0.75</td>
</tr>
<tr>
<td>7</td>
<td>6g</td>
<td>4-OH 3-OCH3</td>
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<td>58</td>
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<tr>
<td>8</td>
<td>6h</td>
<td>3-NO2</td>
<td>78</td>
<td>67</td>
<td>0.82</td>
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<tr>
<td>9</td>
<td>6i</td>
<td>4-N(CH3)2</td>
<td>118</td>
<td>66</td>
<td>0.63</td>
</tr>
<tr>
<td>10</td>
<td>6j</td>
<td>3,4(OCH3)2</td>
<td>142</td>
<td>56</td>
<td>0.68</td>
</tr>
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</table>
Table No.2: Effect of topical application of 2.5% w/w ointment of synthesized compounds on excision (open) wound model

<table>
<thead>
<tr>
<th>S.No</th>
<th>Group</th>
<th>% Contraction of wound on different days (sq.mm)</th>
<th>Epithelization time in days</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>4th day</td>
<td>8th day</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>12.33 ± 1.45</td>
<td>40.33 ± 2.60</td>
</tr>
<tr>
<td>2</td>
<td>Povidine iodine</td>
<td>44.5 ± 3.72***</td>
<td>82.2 ± 1.14***</td>
</tr>
<tr>
<td>3</td>
<td>6- a</td>
<td>10.43 ± 2.73**</td>
<td>57.26 ± 2.40</td>
</tr>
<tr>
<td>4</td>
<td>6- d</td>
<td>19.86 ± 0.86**</td>
<td>64.16 ± 2.25</td>
</tr>
<tr>
<td>5</td>
<td>6-f</td>
<td>21.0 ± 2.08**</td>
<td>52.0 ± 2.30***</td>
</tr>
<tr>
<td>6</td>
<td>6-h</td>
<td>23.0 ± 2.18**</td>
<td>57.66 ± 1.45***</td>
</tr>
</tbody>
</table>
Figure No.1 (6c): IR Spectrum of N''-(4-methylbenzylidene)-N''''-(tetrazolo[1,5-a]quinolin-4-yl methylidene) thiocarbohydrazide

Figure No.2 (6e): IR Spectrum of N''-(4-fluorobenzylidene)-N''''-(tetrazolo[1,5-a]quinolin-4-yl methylidene) thiocarbohydrazide.
CONCLUSION
From the results it was concluded that the wound healing activity of the compound 6d was found closer (wound contraction 96.93% on day 16\textsuperscript{th} and epithelization time on day 18.33) to that of standard drug povidine iodine (97.80% wound contraction on 16\textsuperscript{th} day and epithelization period on day 17.66), due to the presence of \( p \)-methoxy phenyl ring (electron donating group) in compound 6d might have been favored an significant wound healing activity. However other compounds like 6a, 6h and 6f possed moderate to mild wound healing activity.

ACKNOWLEDGEMENT
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CONFLICT OF INTEREST
We declare that we have no conflict of interest.

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